DOCKET NO: 263192US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
MASAZUMI NISHIKAWA, ET AL.	: EXAMINER: MERCIER, M. S.
SERIAL NO: 10/517,323	:
FILED: DECEMBER 20, 2004	: GROUP ART UNIT: 1615
FOR: ORAL PREVENTIVE/THERAPEUTIC AGENT FOR SKIN DAMAGE CONTAINING DIACYLGLYCERYL ETHER	:

APPEAL BRIEF

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Further to the June 8, 2010, Notice of Appeal, this is an Appeal from the January 8, 2010, Final Rejection.

I. REAL PARTY IN INTEREST

The real party in interest in this appeal is Maruha Nichiro Seafoods, Inc., Tokyo, Japan.

II. RELATED APPEALS AND INTERFERENCES

Appellants, Appellants' legal representative and the assignee are aware of no appeals, interferences, or judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

III. STATUS OF CLAIMS

Claims 11-24 are pending and stand rejected.

Claims 1-17, 20-21, 23-24, and 37-38 have been canceled.

The rejections of claims 18-19, 22, 25-36, and 39-41 are being appealed.

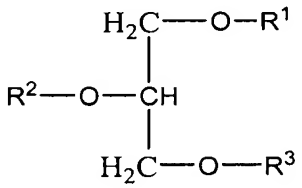
IV. STATUS OF AMENDMENTS

An Amendment After Final Rejection was filed on April 8, 2010.

In the May 4, 2010 Advisory Action, the Examiner indicated that the amendments set forth in the April 4, 2010 Amendment After Final Rejection were entered for the purposes of this appeal.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 18 is directed to a method of reducing at least one skin damage in a subject in need thereof. *See* instant specification, page 1, lines 2-3. The method comprises orally administering to the subject in need thereof a composition comprising diacylglyceryl ether represented by the formula (I), triglyceride, and squalene; in an-amount sufficient to reduce the at least one skin damage:



wherein R^1 denotes C_{12-24} aliphatic hydrocarbon group having a degree of unsaturation of between 0 and 2; R^2 denotes C_{12-24} acyl group having a degree of unsaturation of between 0 and 6; and R^3 denotes C_{12-24} acyl group having a degree of unsaturation of

between 0 and 6. *See* instant specification, page 3, line 17 - page 4, line 4. The at least one skin damage is selected from the group consisting of formation of skin cancer induced by ultraviolet light, formation of pigmented spots induced by ultraviolet light, formation of freckles induced by ultraviolet light, the formation of wrinkles induced by ultraviolet light, the formation of verrucae induced by ultraviolet light, and the formation of erythema induced by ultraviolet light. *See* instant specification, page 10, line 11 - page 11, line 1; and page 12 lines 3-14. Claims 19, 22, 25-36, and 39-41 depend directly or indirectly from claim 18. *See* claims 19, 22, 25-36, and 39-41.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Rejection Under 35 U.S.C. §103

Claims 18-19, 22, 25-36 and 39-41 are rejected under 35 U.S.C. §103(a) over Brohuit (EP 0321428) in view of Ecomer (product information sheet).

VII. ARGUMENT

Appellants submit that the outstanding rejections should be reversed for the following reasons.

A. Rejection Under 35 U.S.C. §103

As indicated above, claims 18-19, 22, 25-36 and 39-41 are rejected under 35 U.S.C. §103(a) over Brohuit (EP 0321428) in view of Ecomer Product Information Sheet.

Independent Claim 18 of the present application is drawn to a method of treating at least one skin damage by orally administering a composition that comprises a diacylglyceryl ether of formula (I), a triglyceride, and *squalene*. Brohuit does not disclose or suggest the use of squalene, and Ecomer teaches *away* from use of squalene in a pharmaceutical preparation due to its toxicity. Accordingly, and for at least the reason that the Examiner has failed to establish motivation to combine all elements of claim 18 the rejection should be REVERSED.

Brohuit discloses the use of glycerol ethers in a pharmaceutical preparation for the treatment of diseases affecting epidermal growth. The pharmaceutical preparation used by Brohuit for said treatment, both in the disclosure and in all experimental examples, is Ecomer shark liver oil. (*See* Abstract; Examples 1-3, col. 2, lines 31-59). This fact is supported by the Examiner in the Office Action of June 25, 2009. (*See* page 3, lines 7-10). The Ecomer Product Information Sheet cited by the Examiner in the Office Action of June 25, 2009, does not include squalene. Importantly, and quite to the contrary:

Squalene and excessive amounts of Vitamins A and D have been **removed** from this product since the manufacturer considers them toxic in high doses.

See Ecomer Monograph by American Nutriceuticals; page 2, paragraph 3, as cited in the Request for Reconsideration, filed on June 20, 2010. (emphasis added).

Accordingly, the pharmaceutical composition of Brohuit does not comprise squalene, and does not suggest that squalene should be used. The Examiner has failed to identify where in the cited references the claimed element squalene is disclosed. Considering this deficiency, it is clear that the Examiner has failed to meet her burden to establish a *prima facie* case of obviousness. On this basis alone the rejection cannot be sustained.

Applicants note that Ecomer materially "teaches away" from the use of a pharmaceutical composition comprising squalene. (A *prima facie* case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention. *See* M.P.E.P. § 2144.05(III)). The Ecomer Monograph, which is published by American Nutriceuticals and describes the composition of Ecomer, explicitly states that: "*Squalene* and excessive amounts of vitamins A and D have been *removed* from this product [Ecomer] since the manufacturer considers them *toxic* in high doses." (*See* Ecomer Monograph by American Nutriceuticals; page 2, paragraph 3, as cited in the Request for Reconsideration, filed on June 20, 2010). Accordingly, even if a *prima facie* case of obviousness were established, which it is not, the fact that the manufacturer of Ecomer removed squalene from the pharmaceutical composition used in Brohuit because it was considered toxic provides more than sufficient "teaching away" to rebut the same.

Furthermore, Applicants note that Claim 18 is directed to a method of reducing at least one skin damage selected from the group consisting of formation of skin cancer induced by ultraviolet (UV), formation of pigmented spots induced by UV, formation of freckles induced by UV, formation of wrinkles induced by UV, formation of verrucae induced by UV, and formation of erythema induced by UV. Brohuit does not describe or support the reduction of these specific skin damages. Brohuit discloses the use of glycerol ether for treatment of diseases pertaining to epidermal growth, and Brohuit does not provide or reference any experimental support for any disease other than psoriasis.

Brohuit discloses five medical effects of glycerol ethers:

(1) Oral administration of glycerol ethers by radition therapy reduces the number of harmful radiation effects; leucopenia and thrombocytopenia is partly or fully prevented;

(2) Glycerol ethers improve the bodily immunity defense mechanism;

(3) Glycerol ethers beneficially influence diseases depending upon epidermal growth, and specifically have beneficial influence on the so-called epidermal growth factor;

(4) Psoriasis can be improved by administering Ecomer. This effect is confirmed in Examples 1 to 3;

(5) Glycerol ethers can partly prevent burns caused by intensive sunray.

The medical effects recited in items (1) -(5) do not support the oral administration of diacylglyceryl ether to reduce the skin damages according to the present invention.

The medical effect disclosed in item (1) is irrelevant to the claimed reduction of skin damages.

The medical effect disclosed in item (2) is also irrelevant to the claimed reduction of skin damage. There is no suggestion in Brohuit that a substance effective in improving the bodily immunity defense mechanism is also effective in the a method of method of reducing at least one skin damage selected from the group consisting of formation of skin cancer induced by ultraviolet light, formation of pigmented spots induced by ultraviolet light, formation of freckles induced by ultraviolet light, formation of wrinkles induced by ultraviolet light, formation of verrucae induced by ultraviolet light, and formation of erythema induced by ultraviolet light.

With respect to item (3), the Examiner asserts that "it would have been obvious...to have used the composition of Brohuit for the treatment of UV light induced skin damage since it is disclosed that ethers beneficially influence diseases depending upon epidermal growth" (*See* Official Action of June 25, 2009, page 4). This reasoning is flawed.

Applicants note a large number of studies have been carried out in the field of diseases depending upon epidermal growth, and to Applicants' knowledge, none of these studies suggests that reduction in the skin damages of Claim 18 depend on regulating epidermal growth. An exemplary article from these studies was enclosed with the Amendment and Request for Reconsideration filed April 8, 2010. (*See* P.I. Chen et al, Journal of Biological Chemistry, 2009). The Chen article does not describe or suggest reduction in skin damage as claimed in present Claim 18, even though the article is directed to epidermal growth factors. Accordingly, Applicants maintain that Brohuit does not disclose or support the reduction of the specific skin damages of claim 18, and thus the Examiner has also failed to establish a *prima facie* case of obviousness with respect to the claimed damages.

With respect to item (4), Applicants note that psoriasis is not caused by UV radiation. Since psoriasis is irrelevant to UV radiation, the disclosure of Brohuit that psoriasis can be treated by orally administering Ecomer does not teach or suggest that skin damage caused by UV can also be reduced by orally administering Ecomer.

With respect to item (5), the sentence "burns caused by intensive sunray can be partly prevented" at col. 1, lines 57-58, does not specify whether or not such efficacy is achieved when the glycerol ethers are orally administered. It is obvious that the phrase "oral administration" on line 56 of col. 1 refers only to the treatment of severe haemmorhoids. Thus, the Brohuit document does not disclose that burns caused by intensive sunray can be partially prevented by oral administration of the glycerol ethers. The readers of Brohuit would understand that the sentence at col. 1, lines 57-58 refers to a "transdermal administration" not an "oral administration," since burns are usually treated by transdermal administration of a medicament.

This same rational applies for items (2) and (3) above. Brohuit does not disclose that the bodily immunity defense mechanism is improved by oral administration of the glycerol ethers, nor does it disclose that oral administration of glycerol ethers causes beneficial influence on the epidermal growth factor.

Finally, although Brohuit briefly discloses that "[b]urns caused by intensive sunray can be partly prevented," Brohuit is not enabled for reducing the Claim 18 UV light induced skin conditions. (*See* column 1, lines 57-58). Furthermore, the prevention of burns caused by intensive sunray does not suggest the reduction of UV induced skin cancer, pigmented spots, freckles, verrucae, or erythema. The cited references do not describe or suggest these species, as acknowledged at page 3 of the Official Action. Nevertheless, the Office at page 5 of the Official Action, asserts that "since the prior art discloses the composition can be used to treat UV induced skin damage, it would have been obvious to...attempt treatment utilizing the composition for the instantly claimed disorders since they all arise from the same origin." The Office's reasoning is flawed.

First of all, the Office has not specifically pointed out where Brouhit describes the genus skin damage caused by UV radiation. This is because Brouhit does not use the term skin damage caused by UV radiation. The Office assumes a genus not present in Brouhit. Further, the Office has provided no motivation for selecting the specifically claimed skin damage species from a (non-present) broad genus of UV induced skin damage and has shown no reasonable expectation of success in making the selection. For example, the Office has not shown the cited references are enabled for every species in the broad genus of UV induced skin damage, and this weighs against any reasonable expectation of success. Further, Brohuit provides no experimental support for any disease other than psoriasis, and refers to no additional references. Psoriasis is a suspected autoimmune disease, and factors "that may aggravate psoriasis include stress, withdrawal of systemic corticosteroid, excessive alcohol

consumption, and smoking." (*See* http://en.wikipedia.org/wiki/Psoriasis). "It has long been recognized that daily, short, non-burning exposure to sunlight helped to clear or improve psoriasis in some patients" (emphasis added). Indeed, Wikipedia describes that: "It was during the early part of the 20th century that it was recognized that for psoriasis the therapeutic property of sunlight was due to the wavelengths classified as ultraviolet (UV) light" (emphasis added). Thus, far from causing psoriasis, UV light exposure to the skin can be therapeutic for treating psoriasis (emphasis added). Thus, treating psoriasis, a disease that can be treated by exposure to UV light waves, and the only disease state Brohuit may be enabled for, cannot possibly suggest, motivate or enable orally administering glycerol ethers to reduce the claimed conditions induced by UV light.

Borhult and Ecomer fail to disclose or suggest both *squalene* and oral administration of diacylglyceryl ether to reduce at least one of the specific *skin damages* of claim 18. Accordingly, the Examiner has failed to establish a *prima facie* case of obviousness and Applicants request withdrawal of the 103 rejection of claim 18 over Brohuit in view of Ecomer.

Claims 18 is not obvious over Brohuit in view of Ecomer. Claims 19, 22, 25-36, and 39-41 depend directly or indirectly from claim 18 and, thus, also are not obvious over Brohuit in view of Ecomer. Accordingly, reversal of the rejection is respectfully requested.

VIII. CONCLUSION

For the above reasons, it is respectfully requested that all outstanding rejections of the pending claims be REVERSED.

Respectfully submitted,

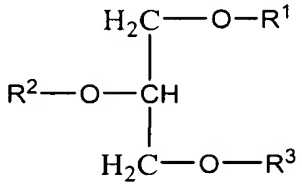
OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, L.L.P.

Anne L. St. Martin
Attorney of Record
Registration No. 65,779

CLAIMS APPENDIX

Claims 1-17 (Canceled).

Claim 18 (Previously Presented): A method of reducing at least one skin damage in a subject in need thereof, comprising orally administering to the subject in need thereof a composition comprising diacylglyceryl ether represented by the formula (I), triglyceride, and squalene; in an-amount sufficient to reduce the at least one skin damage:



wherein R^1 denotes C_{12-24} aliphatic hydrocarbon group having a degree of unsaturation of between 0 and 2; R^2 denotes C_{12-24} acyl group having a degree of unsaturation of between 0 and 6; and R^3 denotes C_{12-24} acyl group having a degree of unsaturation of between 0 and 6, and

wherein the at least one skin damage is selected from the group consisting of formation of skin cancer induced by ultraviolet light, formation of pigmented spots induced by ultraviolet light, formation of freckles induced by ultraviolet light, the formation of wrinkles induced by ultraviolet light, the formation of verrucae induced by ultraviolet light, and the formation of erythema induced by ultraviolet light.

Claim 19 (Previously Presented): The method of claim 18, wherein the diacylglyceryl ether, in the composition, is orally administered at a dosage of between 10 mg and 5000 mg per day.

Claims 20-21 (Canceled).

Claim 22 (Previously Presented): The method of claim 18, wherein the composition is provided in the form of a processed food.

Claims 23-24 (Canceled).

Claim 25 (Previously Presented): The method of claim 18, wherein the diacylglyceryl ether, in the composition, is orally administered at a dosage of between 100 mg and 2000 mg per day.

Claim 26 (Previously Presented): The method of claim 18, wherein the at least one diacylglyceryl ether, in the composition, is orally administered at a dosage of between 500 mg and 2000 mg per day.

Claim 27 (Previously Presented): The method of claim 18, wherein the composition is provided in liquid form.

Claim 28 (Previously Presented): The method of claim 27, wherein the liquid form is a suspension, emulsion, syrup, or elixir.

Claim 29 (Previously Presented): The method of claim 18, wherein the composition is provided in the form of a tablet, sustained-release tablet, granule, fine-grained agent, chewable tablet, sublingual tablet, or gum.

Claim 30 (Previously Presented): The method of claim 18, wherein the oral administering is carried out once a day or at several separate instances a day.

Claim 31 (Previously Presented): The method of claim 18, wherein the composition further comprises at least one further component selected from the group consisting of an excipient, a binder, a disintegrating agent, a surfactant, a lubricant, an agent for promoting flowability, a pH regulator, an absorption retarder, an antioxidant, an antiseptic, a corrigent, a colorant, an odorant, and mixtures thereof.

Claim 32 (Previously Presented): The method of claim 18, wherein the at least one skin damage is the formation of skin cancer induced by ultraviolet light.

Claim 33 (Previously Presented): The method of claim 18, wherein the at least one skin damage is the formation of freckles induced by ultraviolet light.

Claim 34 (Previously Presented): The method of claim 18, wherein the at least one skin damage is the formation of pigmented spots induced by ultraviolet light.

Claim 35 (Previously Presented): The method of claim 18, wherein the at least one skin damage is the formation of verrucae induced by ultraviolet light.

Claim 36 (Previously Presented): The method of claim 18, wherein the at least one skin damage is the formation of erythema induced by ultraviolet light.

Claims 37-38 (Canceled).

Claim 39 (Previously Presented): The method of claim 18, wherein the composition comprises 66.4% diacylglyceryl ether represented by the formula (I), 26.3% triglyceride, and 7.5% squalene.

Claim 40 (Previously Presented): The method of claim 39, wherein the composition is prepared from degummed shark liver oil.

Claim 41 (Previously Presented): The method of claim 18, wherein the composition is prepared from degummed shark liver oil.

EVIDENCE APPENDIX

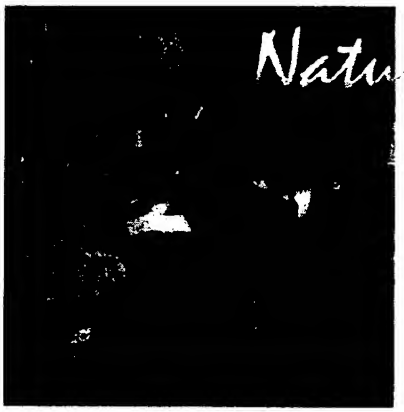
Ecomer Monograph by American Nutriceuticals; page 2, paragraph 3

http://en.wikipedia.org/wiki/Psoriasis

P.I. Chen et al, Journal of Biological Chemistry, 2009

RELATED PROCEEDINGS APPENDIX

None.



Natural Health Consultants

"A Natural Pharmacy"

888 852-4993

by American Nutriceuticals

WHAT IS IT?

Ecomer™ Shark Liver Oil is derived from shark liver oil from the Greenland shark which lives in cold, deep, northern waters. This oil is a rich source of squalene, alkylglycerol (glycerol ether lipids or AKG,) omega-3 EFA's, and vitamins A, D and E. This shark has the highest concentrations of these nutrients and lives where pollutants are less likely to be concentrated in the liver.

Each 250 mg capsule contains 50 mg of alkylglycerols. Other ingredients are gelatin and glycerin.

Alkylglycerols are also concentrated in mother's milk where it performs an immune stimulating function. Squalene and excessive amounts of Vitamins A and D have been removed from this product since the manufacturer considers them toxic in high doses.

WHAT DOES IT DO?

Shark liver oil stimulates blood leukocyte and thrombocyte production as well as activating macrophage (stimulated to produce over 50 immune related substances) and anti-tumor activity. In the case of tumors it lowered the ability of the cells to reproduce and inhibited their ability to invade healthy cells. Protein kinase C, an essential step in cancer cell growth, is inhibited or stopped by alkylglycerols.

Among its properties are:

1. Immune booster. Alkylglycerol normalizes bone marrow function but does not overstimulate it.

2. Decreases platelet aggregation and abnormal clots.

3. Anti-inflammatory.

4. Vasodilator.

5. Beneficial effects on skin and mucous membranes.

6. Antioxidant. The substances in shark liver oil can act as an intracellular antioxidant, penetrating where most damage occurs.

CAUTIONS

No toxicities have been noted. High doses over 30 days have been rarely noted to cause an excess in platelets and a tendency to clot. This can be counteracted with Omega-3 oils.

See Disclaimer.

DOSE

Take one or two capsules 2 or 3 times daily or as directed by a health practitioner.

NHC sells cartons of 120 capsules of **Ecomer 250 mg** for **$25.20**. The suggested retail price is $28.00.

1 Add To Shopping Cart

View Shopping Cart

CI

These statements have not been evaluated by the U.S. Food & Drug Administration (FDA).
The products discussed are not intended to diagnose, treat, cure, or prevent any disease.

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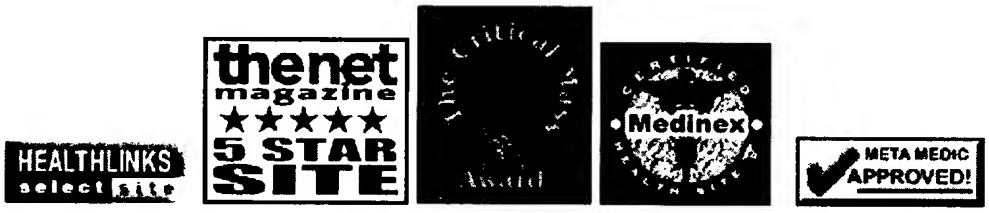
Gerry Wolke, RPh.



Psoriasis

From Wikipedia, the free encyclopedia

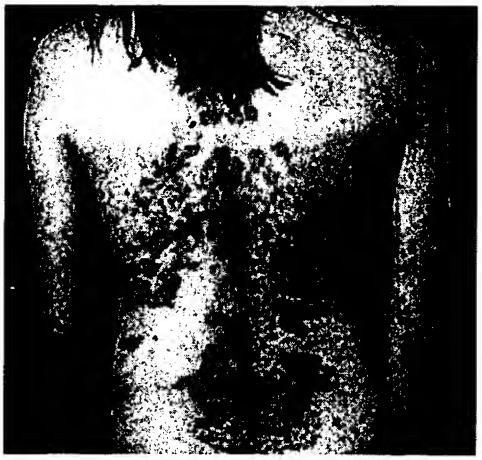
Psoriasis (pronounced /səˈraɪ.əsɨs/) is a chronic, autoimmune disease that appears on the skin. It occurs when the immune system sends out faulty signals that speed up the growth cycle of skin cells. Psoriasis is not contagious.[1] It commonly causes red, scaly patches to appear on the skin, although some patients have no dermatological symptoms. The scaly patches commonly caused by psoriasis, called psoriatic plaques, are areas of inflammation and excessive skin production. Skin rapidly accumulates at these sites which gives it a silvery-white appearance. Plaques frequently occur on the skin of the elbows and knees, but can affect any area including the scalp, palms of hands and soles of feet, and genitals. In contrast to eczema, psoriasis is more likely to be found on the outer side of the joint.

The disorder is a chronic recurring condition that varies in severity from minor localized patches to complete body coverage. Fingernails and toenails are frequently affected (psoriatic nail dystrophy) and can be seen as an isolated symptom. Psoriasis can also cause inflammation of the joints, which is known as psoriatic arthritis. Ten to fifteen percent of people with psoriasis have psoriatic arthritis.[2],

The cause of psoriasis is not fully understood, but it is believed to have a genetic component and local psoriatic changes can be triggered by an injury to the skin known as Koebner phenomenon[*citation needed*]. Various environmental factors have been suggested as aggravating to psoriasis including stress, withdrawal of systemic corticosteroid, excessive alcohol consumption, and smoking but few have shown statistical significance.[3] There are many treatments available, but because of its chronic recurrent nature psoriasis is a challenge to treat.

Psoriasis

Classification and external resources



A person whose back and arms are affected by psoriasis

ICD-10	L40.
ICD-9	696
OMIM	177900
DiseasesDB	10895
MedlinePlus	000434
eMedicine	emerg/489 Dermatology:derm/365 plaque derm/361 guttate derm/363 nails derm/366 pustular Arthritis derm/918 Radiology radio/578 Physical Medicine pmr/120
MeSH	D011565

Contents

Classification

The symptoms of psoriasis can manifest in a variety of forms. Variants include plaque, pustular, guttate and flexural psoriasis. This section describes each type (with ICD-10 code [5]).[4]

Psoriasis is a chronic relapsing disease of the skin that may be classified into nonpustular and pustular types as follows[5]:414:

Nonpustular psoriasis

- Psoriasis vulgaris (Chronic stationary psoriasis, Plaque-like psoriasis). **Plaque psoriasis (*psoriasis vulgaris*) (L40.0)** is the most common form of psoriasis. It affects 80 to 90% of people with psoriasis. Plaque psoriasis typically appears as raised areas of inflamed skin covered with silvery white scaly skin. These areas are called plaques.

- Psoriatic erythroderma (Erythrodermic psoriasis). **Erythrodermic psoriasis (L40.85)** involves the widespread inflammation and exfoliation of the skin over most of the body surface. It may be accompanied by severe itching, swelling and pain. It is often the result of an exacerbation of unstable plaque psoriasis, particularly following the abrupt withdrawal of systemic treatment. This form of psoriasis can be fatal, as the extreme inflammation and exfoliation disrupt the body's ability to regulate temperature and for the skin to perform barrier functions.[6]

Pustular psoriasis

Pustular psoriasis (L40.1-3, L40.82) appears as raised bumps that are filled with non-infectious pus (pustules). The skin under and surrounding the pustules is red and tender. Pustular psoriasis can be localised, commonly to the hands and feet (palmoplantar pustulosis), or generalised with widespread patches occurring randomly on any part of the body.

- Generalized pustular psoriasis (Pustular psoriasis of von Zumbusch)
- Pustulosis palmaris et plantaris (Persistent palmoplantar pustulosis, Pustular psoriasis of the Barber type, Pustular psoriasis of the extremities)
- Annular pustular psoriasis
- Acrodermatitis continua
- Impetigo herpetiformis

Other psoriasis

Additional types of psoriasis include[7]:191-197:

- Drug-induced psoriasis
- Inverse psoriasis. **Flexural psoriasis (inverse psoriasis) (L40.83-4)** appears as smooth inflamed patches of skin. It occurs in skin folds, particularly around the genitals (between the thigh and groin), the armpits, under an overweight stomach (pannus), and under the breasts (inframammary fold). It is aggravated by friction and sweat, and is vulnerable to fungal infections.
- Napkin psoriasis
- Seborrheic-like psoriasis

Guttate psoriasis (L40.4) is characterized by numerous small, scaly, red or pink, teardrop-shaped lesions. These numerous spots of psoriasis appear over large areas of the body, primarily the trunk, but also the limbs, and scalp. Guttate psoriasis is often preceded by a streptococcal infection, typically streptococcal pharyngitis. The reverse is not true.

Nail psoriasis (L40.86) produces a variety of changes in the appearance of finger and toe nails. These changes include discolouring under the nail plate, pitting of the nails, lines going across the nails, thickening of the skin under the nail, and the loosening (onycholysis) and crumbling of the nail.

Psoriatic arthritis (L40.5) involves joint and connective tissue inflammation. Psoriatic arthritis can affect any joint but is most common in the joints of the fingers and toes. This can result in a sausage-shaped swelling of the fingers and toes known as dactylitis. Psoriatic arthritis can also affect the hips, knees and spine (spondylitis). About 10-15% of people who have psoriasis also have psoriatic arthritis.

Signs and symptoms

Plaque of psoriasis

Plaque of psoriasis

An arm covered with plaque psoriasis

Psoriasis of a fingernail

Quality of life

Psoriasis has been shown to affect health-related quality of life to an extent similar to the effects of other chronic diseases such as depression, myocardial infarction, hypertension, congestive heart failure or type 2 diabetes.[8] Depending on the severity and location of outbreaks, individuals may experience significant physical discomfort and some disability. Itching and pain can interfere with basic functions, such as self-care, walking, and sleep. Plaques on hands and feet can prevent individuals from working at certain occupations, playing some sports, and caring for family members or a home. Plaques on the scalp can be particularly embarrassing as flaky plaque in the hair can be mistaken for dandruff. Medical care can be costly and time-consuming and can interfere with an employment or school schedule.

Individuals with psoriasis may also feel self-conscious about their appearance and have a poor self-image that stems from fear of public rejection and psychosexual concerns.[*citation needed*] Psychological distress can lead to significant depression and social isolation.

In a 2008 National Psoriasis Foundation survey of 426 psoriasis sufferers, 71 percent reported that the disease was a significant problem in everyday life. More than half reported significant feelings of self-consciousness (63 percent) and embarrassment (58 percent). More than one-third said they avoided social activities and limited dating or intimate interactions.[9]

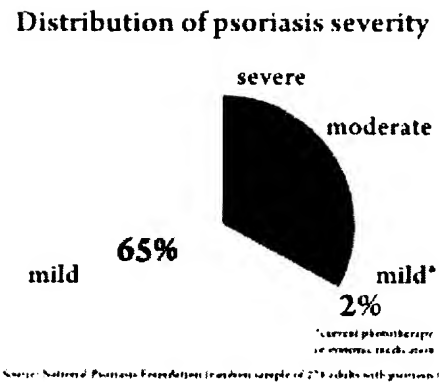
Many tools exist to measure quality of life of patients with psoriasis and other dermatalogical disorders. Clinical research has indicated that individuals often experience a diminished quality of life.[10] A 2009 study looked at the impact of psoriasis by using interviews with dermatologists and exploring patients viewpoint. It found that in cases of mild and severe psoriasis, itch contributed most to the diminished health-related quality of life (HRQoL).[11]

Severity

Psoriasis is usually graded as mild (affecting less than 3% of the body), moderate (affecting 3-10% of the body) or severe.[*citation needed*] Several scales exist for measuring the severity of psoriasis. The degree of severity is generally based on the following factors: the proportion of body surface area affected; disease activity (degree of plaque redness, thickness and scaling); response to previous therapies; and the impact of the disease on the person.

The Psoriasis Area Severity Index (PASI) is the most widely used measurement tool for psoriasis. PASI combines the assessment of the severity of lesions and the area affected into a single score in the range 0

(no disease) to 72 (maximal disease).[12] Nevertheless, the PASI can be too unwieldy to use outside of trials, which has led to attempts to simplify the index for clinical use.[13]



Pie chart showing the distribution of severity among people with psoriasis

Cause

The cause of psoriasis is not fully understood. There are two main hypotheses about the process that occurs in the development of the disease. The first considers psoriasis as primarily a disorder of excessive growth and reproduction of skin cells. The problem is simply seen as a fault of the epidermis and its keratinocytes. The second hypothesis sees the disease as being an immune-mediated disorder in which the excessive reproduction of skin cells is secondary to factors produced by the immune system. T cells (which normally help protect the body against infection) become active, migrate to the dermis and trigger the release of cytokines (tumor necrosis factor-alpha TNFα, in particular) which cause inflammation and the rapid production of skin cells. It is not known what initiates the activation of the T cells.

The immune-mediated model of psoriasis has been supported by the observation that immunosuppressant medications can clear psoriasis plaques. However, the role of the immune system is not fully understood, and it has recently been reported that an animal model of psoriasis can be triggered in mice lacking T cells.[14] Animal models, however, reveal only a few aspects resembling human psoriasis.

Compromised skin barrier function has a role in psoriasis susceptibility.[15]

Psoriasis is a fairly idiosyncratic disease. The majority of people's experience of psoriasis is one in which it may worsen or improve for no apparent reason. Studies of the factors associated with psoriasis tend to be based on small (usually hospital based) samples of individuals. These studies tend to suffer from representative issues, and an inability to tease out causal associations in the face of other (possibly unknown) intervening factors. Conflicting findings are often reported. Nevertheless, the first outbreak is sometimes reported following stress (physical and mental), skin injury, and streptococcal infection. Conditions that have been reported as accompanying a worsening of the disease include infections, stress, and changes in season and climate. Certain medicines, including lithium salt, beta blockers and the Antimalarial drug chloroquininine have been reported to trigger or aggravate the disease. Excessive alcohol consumption, smoking and obesity may exacerbate psoriasis or make the management of the condition difficult or perhaps these comorbidities are effects rather than causes.[16][17] Hairspray, some face creams and hand lotions, can also cause an outbreak of psoriasis.[*citation needed*] In 1975, Stefania Jablonska and collaborators advanced a new theory that special antibodies tend to break through into the lower layers of the skin and set up a complex series of chemical reactions.[18]

Individuals suffering from the advanced effects of the Human immunodeficiency virus, or HIV, often exhibit psoriasis.[19] This presents a paradox to researchers as traditional therapies that reduce T-cell counts generally cause psoriasis to improve. Yet, as CD4-T-cell counts decrease with the progression of HIV, psoriasis worsens.[20] In addition, HIV is typically characterized by a strong Th2 cytokine profile, whereas psoriasis vulgaris is characterized by a strong Th1 secretion pattern.[21] It is hypothesized that the diminished CD4-T-Cell presence causes an over-activation of CD8-T-Cells, which are responsible for the exacerbation of psoriasis in HIV positive patients. It is important to remember that most

individuals with psoriasis are otherwise healthy and the presence of HIV accounts for less than 1% of cases. The prevalence of psoriasis in the HIV positive population ranges from 1 to 6 percent, which is about 3 times higher than the normal population.[22] Psoriasis in AIDS sufferers is often severe, and untreatable with conventional therapy.[23]

Psoriasis occurs more likely in dry skin than oily or well-moisturized skin, and specifically after an external skin injury such as a scratch or cut (see Koebner phenomenon). This is believed to be caused by an infection, in which the infecting organism thrives under dry skin conditions with minimal skin oil, which otherwise protects skin from infections. The case for psoriasis is opposite to the case of athlete's foot, which occurs because of a fungus infection under wet conditions as opposed to dry in psoriasis. This infection induces inflammation, which causes the symptoms commonly associated with psoriasis, such as itching and rapid skin turnover, and leads to drier skin as the infecting organism absorbs the moisture that would otherwise go to the skin. To prevent dry skin and reduce psoriasis symptoms, it is advised to not use shower scrubs, as they not only damage skin by leaving tiny scratches, they also scrape off the naturally occurring skin oil. It is recommended to use talc powder after washing as that helps absorb excess moisture which would otherwise go to the infecting agent. Additionally, moisturizers can be applied to moisturize the skin, and lotions used to promote skin oil gland functions. [*citation needed*]

Genetic factors

Psoriasis has a large hereditary component, and many genes are associated with it, but it is not clear how those genes work together. Most of them involve the immune system, particularly the major histocompatibility complex (MHC) and T cells. The main value of genetic studies is that they identify molecular mechanisms and pathways for further study and potential drug targets.[24]

Classic genomewide linkage analysis has identified nine locations (loci) on different chromosomes that are associated with psoriasis. They are called psoriasis susceptibility 1 through 9 (PSORS1 through PSORS9). Within those loci are genes. Many of those genes are on pathways that lead to inflammation. Certain variations (mutations) of those genes are commonly found in psoriasis.[24]

The major determinant is PSORS1, which probably accounts for 35-50% of its heritability. It controls genes that affect the immune system or encode proteins that are found in the skin in greater amounts in psoriasis. PSORS1 is located on chromosome 6 in the MHC, which controls important immune functions. Three genes in the PSORS1 locus have a strong association with psoriasis vulgaris: HLA-C variant HLA-Cw6, which encodes a MHC class I protein; CCHCR1, variant WWC, which encodes a coiled protein that is overexpressed in psoriatic epidermis; and CDSM, variant allele 5, which encodes corneodesmosin, which is expressed in the granular and cornified layers of the epidermis and upregulated in psoriasis.[24]

Genomewide association scans have identified other genes which are altered to characteristic variants in psoriasis. Some of these genes express inflammatory signal proteins, which affect cells in the immune system that are also involved in psoriasis. Some of these genes are also involved in other autoimmune diseases.[24]

Two major genes under investigation are IL12B on chromosome 5q which expresses interleukin-12B; and IL23R on chromosome 1p which expresses the interleukin-23 receptor, and is involved in T cell differentiation. T cells are involved in the inflammatory process that leads to psoriasis.[24]

These genes are on the pathway that ends up upregulating tumor necrosis factor-α and nuclear factor κB,

two genes that are involved in inflammation.[24]

Immunological factors

In psoriasis, immune cells move from the dermis to the epidermis, where they stimulate skin cells (keratinocytes) to proliferate. Psoriasis does not seem to be a true autoimmune disease.[24] In an autoimmune disease, the immune system confuses an outside antigen with a normal body component, and attacks them both. But in psoriasis, the inflammation doesn't seem to be caused by outside antigens (although DNA does have an immunostimulatory effect). Researchers have identified many of the immune cells that are involved in psoriasis, and the chemical signals they send to each other to coordinate inflammation. At the end of this process, immune cells such as dendritic cells and T cells move from the dermis to the epidermis, secreting chemical signals, such as tumor necrosis factor-α, interleukin-1β, and interleukin-6, which cause inflammation, and interleukin-22, which causes keratinocytes to proliferate.[24]

The immune system consists of an innate immune system, and an adaptive immune system.

In the innate system, immune cells have receptors that have evolved to target specific proteins and other antigens which are commonly found on pathogens. In the adaptive immune system, immune cells respond to proteins and other antigens that they may never have seen before, which are presented to them by other cells. The innate system often passes antigens on to the adaptive system. When the immune system makes a mistake, and identifies a healthy part of the body as a foreign antigen, the immune system attacks that protein, as it does in autoimmunity.

In psoriasis, DNA is an inflammatory stimulus. DNA stimulates the receptors on plasmacytoid dendritic cells, which produce interferon-α, an immune stimulatory signal (cytokine). In psoriasis, keratinocytes produce antimicrobial peptides. In response to dendritic cells and T cells, they also produce cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor-α, which signals more inflammatory cells to arrive and produces further inflammation.[24]

Dendritic cells bridge the innate and adaptive immune system. They are increased in psoriatic lesions and induce the proliferation of T cells and type 1 helper T cells. Certain dendritic cells can produce tumor necrosis factor-alpha, which calls more immune cells and stimulates more inflammation. Targeted immunotherapy, and psoralen and ultraviolet A (PUVA) therapy, reduces the number of dendritic cells. [24]

T cells move from the dermis into the epidermis. They are attracted to the epidermis by alpha-1 beta-1 integrin, a signalling molecule on the collagen in the epidermis. Psoriatic T cells secrete interferon-γ and interleukin-17. Interleukin-17 is also associated with interleukin-22. Interleukin-22 induces keratocytes to proliferate.[24]

One hypothesis is that psoriasis involves a defect in regulatory T cells, and in the regulatory cytokine interleukin-10.[24]

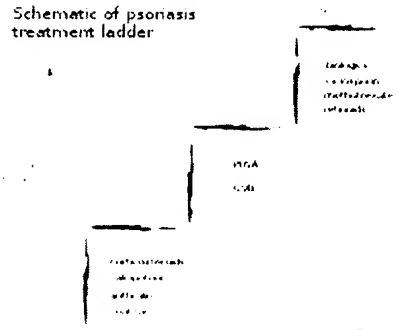
Diagnosis

A diagnosis of psoriasis is usually based on the appearance of the skin. There are no special blood tests or diagnostic procedures for psoriasis. Sometimes a skin biopsy, or scraping, may be needed to rule out other disorders and to confirm the diagnosis. Skin from a biopsy will show clubbed Rete pegs if positive

for psoriasis. Another sign of psoriasis is that when the plaques are scraped, one can see pinpoint bleeding from the skin below (Auspitz's sign).

Management

Research in the past decade has led to "new, highly effective targeted therapies," with phase III data or regulatory approval. They make use of research into how immune cells like T cells and dendrocytes travel, and how they use chemical signals (cytokines) to interact with each other. The drugs follow two strategies: anti-T cell strategies and anticytokine strategies.[24]



Schematic of psoriasis treatment ladder

Two drugs that target T cells are efalizumab and alefacept. Efalizumab (which is no longer marketed) is a monoclonal antibody which blocks the molecules that dendritic cells use to communicate with T cells. It also blocks the adhesion molecules on the endothelial cells that line blood vessels, which attract T cells. However, it suppressed the immune system's ability to control normally harmless viruses, which led to fatal brain infections. Alefacept also blocks the molecules that dendritic cells use to communicate with T cells, and even causes natural killer cells to kill T cells, as a way of controlling inflammation.[24]

Several monoclonal antibodies (MABs) target cytokines, the molecules that cells use to send inflammatory signals to each other. One of the main inflammatory signals in the body is tumor necrosis factor α (TNF-α), and three MABs -- infliximab, adalimumab and etanercept bind to TNF-α. Two more inflammatory signals are interleukin-23 and interleukin-12. A protein chain, p40, is the same on both of those interleukins, and the monoclonal antibody ustekinumab binds to that common protein to interfere with both of them.[24]

There can be substantial variation between individuals in the effectiveness of specific psoriasis treatments. Because of this, dermatologists often use a trial-and-error approach to finding the most appropriate treatment for their patient. The decision to employ a particular treatment is based on the type of psoriasis, its location, extent and severity. The patient's age, sex, quality of life, comorbidities, and attitude toward risks associated with the treatment are also taken into consideration.

In 2008, the FDA approved three new treatment options[25] available to psoriasis patients: 1) Taclonex Scalp, a new topical ointment for treating scalp psoriasis; 2) the Xtrac Velocity excimer laser system, which emits a high-intensity beam of ultraviolet light, can treat moderate to severe psoriasis; and 3) the biologic drug adalimumab (brand name Humira) was also approved to treat moderate to severe psoriasis. Adalimumab had already been approved to treat psoriatic arthritis.

Medications with the least potential for adverse reactions are preferentially employed. If the treatment goal is not achieved then therapies with greater potential toxicity may be used. Medications with significant toxicity are reserved for severe unresponsive psoriasis. This is called the psoriasis treatment ladder.[26] As a first step, medicated ointments or creams, called topical treatments, are applied to the skin. These treatments include non-steroidal creams such as Zithranol-RR, a topical cream containing anthralin. If topical treatment fails to achieve the desired goal then the next step would be to expose the skin to ultraviolet (UV) radiation. This type of treatment is called phototherapy. The third step involves the use of medications which are taken internally by pill or injection. This approach is called systemic treatment.

A 2010 meta-analysis compares the change in Psoriasis Area and Severity Index (PASI) improvement from baseline in 22 trials. The combination therapy for moderate to severe psoriasis using psoralen with ultraviolet A (PUVA) plus acitretin shows a 97.3% PASI improvement from baseline. Therapy limitations need to be taken into consideration in the treatment of moderate to severe psoriasis, such as the increased risk of skin cancer with phototherapy and birth defects with acitretin.[27]

Over time, psoriasis can become resistant to a specific therapy. Treatments may be periodically changed to prevent resistance developing (tachyphylaxis) and to reduce the chance of adverse reactions occurring. This is called treatment rotation.

Antibiotics are generally not indicated in routine treatment of psoriasis. However, antibiotics may be employed when an infection, such as that caused by the bacteria Streptococcus, triggers an outbreak of psoriasis, as in certain cases of guttate psoriasis.[*citation needed*]

Cognitive behaviour therapy

A psychological symptom management programme has been reported as being a helpful adjunct to traditional therapies in the management of psoriasis.[28]. In the UK The Psoriasis and Psoriatic Arthritis Alliance (PAPAA) a not-for-profit charity has funded research carried out by the University of Manchester, to develop a symptom management programme called Electronic Targeted Intervention for Psoriasis (eTIPs) using a modified Cognitive Behaviour Therapy model. This research follows research by Fortune D G et al.[29] on psychological stress, distress and disability in patients with psoriasis.

Topical treatment

Bath solutions and moisturizers, mineral oil, and petroleum jelly may help soothe affected skin and reduce the dryness which accompanies the build-up of skin on psoriatic plaques. Medicated creams and ointments applied directly to psoriatic plaques can help reduce inflammation, remove built-up scale, reduce skin turn over, and clear affected skin of plaques. Ointment and creams containing coal tar, dithranol (anthralin), corticosteroids like desoximetasone (Topicort), fluocinonide, vitamin D_3 analogues (for example, calcipotriol), and retinoids are routinely used. Argan oil has also been used with some promising results.[30] The use of the Finger tip unit may be helpful in guiding how much topical treatment to use.[31] The mechanism of action of each is probably different but they all help to normalise skin cell production and reduce inflammation. Activated vitamin D and its analogues are highly effective inhibitors of skin cell proliferation.

The disadvantages of topical agents are variably that they can often irritate normal skin, can be time consuming and awkward to apply, cannot be used for long periods, can stain clothing or have a strong odour. As a result, it is sometimes difficult for people to maintain the regular application of these medications. Abrupt withdrawal of some topical agents, particularly corticosteroids, can cause an aggressive recurrence of the condition. This is known as a rebound of the condition.

Some topical agents are used in conjunction with other therapies, especially phototherapy.

Phototherapy

It has long been recognized that daily, short, non-burning exposure to sunlight helped to clear or improve psoriasis in some patients. Niels Finsen was the first physician to investigate the therapeutic

effects of sunlight scientifically and to use selected portions of the solar spectrum in clinical practice. This became known as phototherapy.

Sunlight contains many different wavelengths of light. It was during the early part of the 20th century that it was recognised that for psoriasis the therapeutic property of sunlight was due to the wavelengths classified as ultraviolet (UV) light.

Ultraviolet wavelengths are subdivided into UVA (380–315 nm) UVB (315–280 nm), and UVC (< 280 nm). Ultraviolet B (UVB) (315–280 nm) is absorbed by the epidermis and has a beneficial effect on psoriasis. There are two types of UVB lamps: Narrowband UVB (311 to 312 nm), and Broadband (Wideband, or "FS" type) UVB (290-320 nm). UVB Broadband is more erythemal and therefore requires shorter exposure time, while UVB Narrowband does not include the spectrum of less than 300 nanometers, allowing much higher doses without erythema, and thus considered safer. The UVB Narrowband lamp was developed by Philips Lighting specifically to match the action spectrum of psoriasis, with a sharp emission peak at 311 nm, to have increased effectiveness compared to broadband lamps.[32] Exposure to UVB several times per week, over several weeks can help people attain a remission from psoriasis. Sometimes it is needed to continue the treatments once a week as maintenance, or the chronic disease will return.

In hospitals, ultraviolet light treatment is frequently combined with topical (coal tar, calcipotriol) or systemic treatment (Retinoids) as there is a synergy in their combination. The Ingram regime involves UVB and the application of anthralin paste. The Goeckerman regime combines coal tar ointment with UVB. Because coal tar includes unknown ingredients that might cause cancer, and is a time intensive treatment, the use of coal tar has fallen out of favor.

Photochemotherapy

Psoralen and ultraviolet A phototherapy (PUVA) combines the oral or topical administration of psoralen with exposure to ultraviolet A (UVA) light. Precisely how PUVA works is not known. The mechanism of action probably involves activation of psoralen by UVA light which inhibits the abnormally rapid production of the cells in psoriatic skin. There are multiple mechanisms of action associated with PUVA, including effects on the skin immune system.

PUVA is associated with nausea, headache, fatigue, burning, and itching. Long-term treatment is associated with squamous cell carcinoma (not with melanoma).

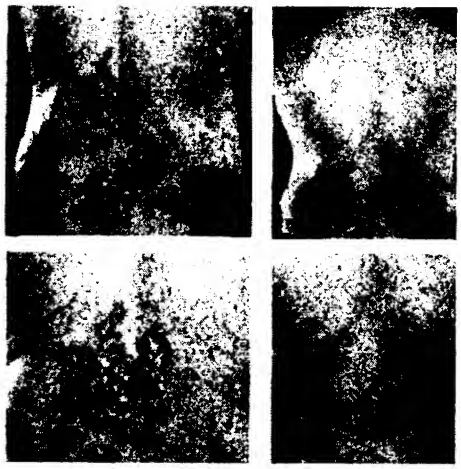
Systemic treatment

Psoriasis that is resistant to topical treatment and phototherapy is treated by medications that are taken internally by pill or injection. This is called systemic treatment. Patients undergoing systemic treatment are required to have regular blood and liver function tests because of the toxicity of the medication. Pregnancy must be avoided for the majority of these treatments. Most people experience a recurrence of psoriasis after systemic treatment is discontinued.

The three main traditional systemic treatments are methotrexate, cyclosporine and retinoids. Methotrexate and cyclosporine are immunosuppressant drugs; retinoids are synthetic forms of vitamin A.

Other additional drugs, not specifically licensed for psoriasis, have been found to be effective. These include the antimetabolites tioguanine, mercaptopurine and fluorouracil, the cytotoxic agents

hydroxyurea and paclitaxel, alkylating agents chlorambucil and cyclophosphamide, some DMARDs like sulfasalazine, colchicine, dapsone, the immunosuppressants mycophenolate mofetil, azathioprine and oral tacrolimus. These have all been used effectively to treat psoriasis when other treatments have failed. Although not licensed in many other countries, fumaric acid esters have also been used to treat severe psoriasis in Germany for over 20 years. There is also some evidence for beneficial effect on psoriasis of insulin-sensitizing drugs (thiazolidinediones like pioglitazone and rosiglitazone, and a more modest effect is described for metformin), somatostatin, bromocriptine, and some lipid-lowering drugs from the group of statines (like simvastatin), and omega-3 fatty acid supplements. For all those drugs it is hypothesised that their antipsoriatic activity comes from their immunomodulatory properties.



Pictures of a patient with psoriasis (and psoriatic arthritis) at baseline and 8 weeks after initiation of infliximab therapy.

There are also case reports and small trials describing beneficial effects of yohimbine (effect is thought to be secondary to its insulin-lowering and growth hormone lowering properties), ketotifen (effect is thought to be secondary to its ability to dampen release of inflammatory mediators) and albuterol (beta-adrenergic agonist).

Antihistamine drugs generally do not help to improve psoriasis lesions, but they may be of use to reduce itching and also are helpful in cases where psoriasis coexists with skin allergy, for example chronic urticaria. Some antihistamines have sedative properties, thus might aid to improve sleep and reduce anxiety in psoriasis patients. Antidepressant medications may help to reduce comorbid depression, anxiety, social isolation, improve sleep and in some cases reduce itching (primarily due to antihistamine effects of tricyclic antidepressants and some SSRIs). Naltrexone, an opioid antagonist, and pregabalin or gabapentin, benzodiazepine anxiolytics are also of use in severe itching.

NSAID drugs generally do not help to improve psoriatic arthritis itself, but they might provide rapid symptomatic relief from pain and swelling.

Biologics are manufactured proteins that interrupt the immune process involved in psoriasis. Unlike generalised immunosuppressant therapies such as methotrexate, biologics focus on specific aspects of the immune function leading to psoriasis. These drugs (interleukin antagonists) are relatively new, and their long-term impact on immune function is unknown, but they have proven effective in treating psoriasis and psoriatic arthritis. They include Amevive, Enbrel, Humira, Remicade and Raptiva. Raptiva was withdrawn by its maker from the US market in April, 2009. Biologics are usually given by self-injection or in a doctor's office. They are very expensive and only suitable for very few patients with severe psoriasis. Ustekinumab (IL-12 and IL-23 blocker) shows hopeful results for psoriasis therapy.

In the United Kingdom in 2005 the British Association of Dermatologists (BAD) published guidelines for use of biological interventions in psoriasis.[33] A UK national register called the BAD Biological Register (BADBIR) has been set up to collect valuable information on side effects and benefits and will be used to inform doctors on how best to use biological agents and similar drugs.

Alternative therapy

Some studies suggest that psoriasis symptoms can be relieved by changes in diet and lifestyle. Fasting periods, low energy diets and vegetarian diets have improved psoriasis symptoms in some studies, and

diets rich in fatty acids from fish oil have also shown beneficial effects.[34] The severity of psoriasis symptoms may also be influenced by lifestyle habits related to alcohol, smoking, weight, sleep, stress and exercise.[35]

Climatotherapy involves the notion that some diseases can be successfully treated by living in a particular climate. Several psoriasis clinics are located throughout the world based on this idea. The Dead Sea is one of the most popular locations for this type of treatment.

Another treatment is ichthyotherapy, which is practised at some spas in Turkey, Croatia, Ireland, Hungary and Serbia. In this therapy, doctor fish are encouraged to feed on the psoriatic skin of people with psoriasis. The fish, which live in outdoor pools, only consume the affected areas of the skin. The outdoor location of the spa may also have a beneficial effect. This treatment can provide temporary relief of symptoms. A revisit to the spas every few months is often required. Treatment in this hot spring has been examined in two small clinical trials, with positive results.[36][37]

Oregon-grape (Mahonia Aquifolium) is said to be effective in the treatment of eczema and psoriasis.[38] [39][40]

Prognosis

Psoriasis is a lifelong condition.[41] There is currently no cure but various treatments can help to control the symptoms. Many of the most effective agents used to treat severe psoriasis carry an increased risk of significant morbidity including skin cancers, lymphoma and liver disease. However, the majority of people's experience of psoriasis is that of minor localized patches, particularly on the elbows and knees, which can be treated with topical medication. Psoriasis can get worse over time but it is not possible to predict who will go on to develop extensive psoriasis or those in whom the disease may appear to vanish. Individuals will often experience flares and remissions throughout their lives. Controlling the signs and symptoms typically requires lifelong therapy.

According to one study,[42] psoriasis is linked to 2.5-fold increased risk for non melanoma skin cancer in men and women, with no preponderance of any specific histologic subtype of cancer. This increased risk could also be attributed to antipsoriatic treatment.

Epidemiology

Psoriasis affects both sexes equally and can occur at any age, although it most commonly appears for the first time between the ages of 15 and 25 years.

The prevalence of psoriasis in Western populations is estimated to be around 2-3%. The prevalence of psoriasis among 7.5 million patients who were registered with a general practitioner in the United Kingdom was 1.5%.[43] A survey[44] conducted by the National Psoriasis Foundation (a US based psoriasis education and advocacy group) found a prevalence of 2.1% among adult Americans. The study found that 35% of people with psoriasis could be classified as having moderate to severe psoriasis.

Around one-third of people with psoriasis report a family history of the disease, and researchers have identified genetic loci associated with the condition. Studies of monozygotic twins suggest a 70% chance of a twin developing psoriasis if the other twin has psoriasis. The concordance is around 20% for dizygotic twins. These findings suggest both a genetic predisposition and an environmental response in

developing psoriasis.[45]

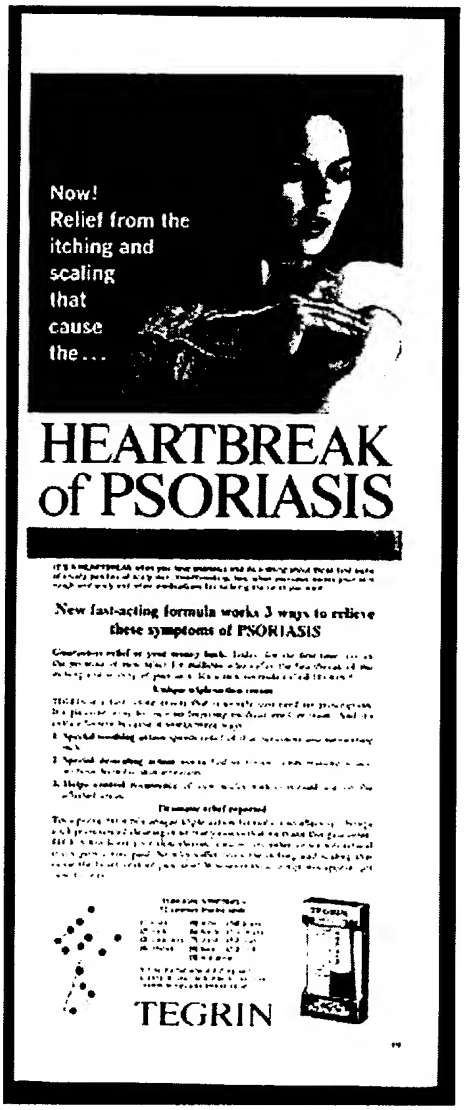
Onset before age 40 usually indicates a greater genetic susceptibility and a more severe or recurrent course of psoriasis.

History

Psoriasis is probably one of the longest known illnesses of humans and simultaneously one of the most misunderstood. Some scholars believe psoriasis to have been included among the skin conditions called tzaraat in the Bible.[46] In more recent times psoriasis was frequently described as a variety of leprosy. The Greeks used the term lepra (λεπρα) for scaly skin conditions. They used the term psora to describe itchy skin conditions. It became known as *Willan's lepra* in the late 18th century when English dermatologists Robert Willan and Thomas Bateman differentiated it from other skin diseases. Leprosy, they said, is distinguished by the regular, circular form of patches, while psoriasis is always irregular. Willan identified two categories: *leprosa graecorum* and *psora leprosa*.[47]

While it may have been visually, and later semantically, confused with leprosy, it was not until 1841 that the condition was finally given the name *psoriasis* by the Viennese dermatologist Ferdinand von Hebra. The name is derived from the Greek word *psora* which means *to itch*.[48]

It was during the 20th century that psoriasis was further differentiated into specific types.



1964 Tegrin advertisement

Historical treatment

The history of psoriasis is littered with treatments of dubious effectiveness and high toxicity. These treatments received brief popularity at particular time periods or within certain geographical regions. The application of cat faeces to red lesions on the skin, for example, was one of the earliest topical treatments employed in ancient Egypt. Onions, sea salt and urine, goose oil and semen, wasp droppings in sycamore milk, and soup made from vipers have all been reported as being ancient treatments.

In the more recent past Fowler's solution, which contains a poisonous and carcinogenic arsenic compound, was used by dermatologists as a treatment for psoriasis during the 18th and 19th centuries. Grenz rays (also called ultrasoft X-rays or Bucky rays) was a popular treatment of psoriasis during the middle of the 20th century. This type of therapy was superseded by ultraviolet therapy.

Undecylenic acid was investigated and used for psoriasis some 40 years ago(cir. 1950~).[49]

All these treatments have fallen out of favour.

Sulphur was fashionable as a treatment for psoriasis in the Victorian and Edwardian eras. It has recently re-gained some credibility as a safe alternative to steroids and coal tar.[*citation needed*]

Research

Historically, agents used to treat psoriasis were discovered by experimentation or by accident. In contrast, current novel therapeutic agents are designed from a better understanding of the immune processes involved in psoriasis and by the specific targeting of molecular mediators. Examples can be seen in the use of biologics which target T cells and TNF inhibitors.

It has been suggested that cannabis might treat psoriasis, due to the anti-inflammatory properties of its cannabinoids, and the regulatory effects of THC on the immune system.[50] The adverse effects of cannabis might be overcome by use of more specific cannabinoid receptor medications,[51] to inhibit keratinocyte proliferation.[52]

Future innovation should see the creation of additional drugs that refine the targeting of immune-mediators further.[53]

Research into antisense oligonucleotides carries the potential to provide novel therapeutic strategies for treating psoriasis.[54]

ABT-874 is a human anti-IL-12 monoclonal antibody being developed by Abbott Laboratories in conjunction with Cambridge Antibody Technology for the treatment of multiple autoimmune diseases including psoriasis. Phase II trials have been completed and showed promising results.[55] Abbott was planning to initiate Phase III trials in 2007.[56]

In 2004, Tas and Avci [57] demonstrated cyclopamine's clinical potential for the treatment of psoriasis and basal cell carcinoma in two preliminary proof of concept studies. By treating 31 psoriatic lesions in 7 patients, these authors asserted that topical cyclopamine was more effective in the clinical and histological clearance of guttate and plaque psoriasis than the topical steroid clobetasol-17 propionate. Furthermore, they demonstrated that concurrent application of cylopamine and clobetasol-17 propionate accelerated regression and clearance of selected lesions greater than cyclopamine alone with clearance times as early as 48 hours.They assert that cyclopamine inhibits the abnormal proliferation of epithelial cells, induces terminal differentiation, and is associated with the decreased presence of inflammatory cells, including CD41 lymphocytes.

On August 27, 2006, scientists led by Jeung-Hoon Lee created in the laboratory synthetic lipids called pseudoceramides which are involved in skin cell growth and could be used in treating skin diseases such as atopic dermatitis, a form of eczema characterized by red, flaky and very itchy skin; psoriasis, and glucocorticoid-induced epidermal atrophy, in which the skin shrinks due to skin cell loss.[58]

On November 17, 2008, scientists led by Yin-Ku Lin of Chang Gung Memorial Hospital and Chang Gung University in Taoyuan, Taiwan, told Reuters by telephone that Indigo naturalis (Qing Dai, 青黛), a dark blue plant used in traditional Chinese medicine, appears to be effective in treating psoriasis. In the latest issue of Archives of Dermatology, they wrote, "The indigo naturalis ointment-treated lesions showed an 81 percent improvement, the (non-medicated) ointment-treated lesions showed a 26 percent improvement."[59]

Talarozole amplifies the effects of retinoic acid by inhibiting its metabolism. As of February 2009, it is undergoing clinical trials.[60]

Psoriasis in children

Psoriasis can affect children. Approximately one third of psoriasis patients report being diagnosed before age 20.[61] Self-esteem and behavior can be affected by the disease. Bullying has been noted in clinical research.[62]

See also

- List of cutaneous conditions
- Psoriatic nails

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External links

- "Questions and Answers about Psoriasis" at National Institute of Arthritis and Musculoskeletal and Skin Diseases
- Psoriasis at National Institute of Arthritis and Musculoskeletal and Skin Diseases

- National Psoriasis Foundation Homepage
- The Psoriasis Association
- The Mayo Clinic
- DermAtlas *7*

Books

- *From Arsenic to Biologicals: A 200 Year History of Psoriasis (Barbara S. Baker)*, ISBN 0-955-16032-4.

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RAB5 ISOFORMS DIFFERENTIALLY REGULATE THE TRAFFICKING AND DEGRADATION OF EPIDERMAL GROWTH FACTOR RECEPTORS

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Running title: Rab5A specifically regulates EGFR degradation pathway

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Ligand-mediated endocytosis is an intricate regulatory mechanism for epidermal growth factor receptor (EGFR) signal transduction. Coordinated trafficking of EGFR ensures its temporal and spatial communication with downstream signaling effectors. We focused our work on Rab5, a monomeric GTPase shown to participate in early stages of the endocytic pathway. Rab5 has three isoforms (A, B and C) sharing more than 90 % of sequence identity. We individually ablated endogenous isoforms in HeLa cells with siRNAs and examined the loss-of-function phenotypes. We found that suppression of Rab5A or 5B hampered the degradation of EGFR, whereas Rab5C depletion had very little effect. The differential delay of EGFR degradation also corresponds with retarded progression of EGFR from early to late endosomes. We investigated the activators/effectors of Rab5A that can potentially separate its potency in EGFR degradation from other isoforms and found that Rin1, a Rab5 exchange factor, preferably associated with Rab5A. Moreover, Rab5A activation is sensitive to EGF stimulation, and suppression of Rin1 diminished this sensitivity. Together with previous work showing that Rin1 interacts with ESCRT-0 complex (Hrs/STAM) to facilitate the degradation of EGFR (1), we hypothesize that the selective association of Rab5A and Rin1 contributes to the dominance of Rab5A in EGFR trafficking, whereas the other isoforms may have major functions unrelated to the EGFR degradation pathway.

Rabs are small-molecular-weight guanine nucleotide binding proteins (G proteins) specialized in regulating different stages of the intracellular membrane trafficking based on their subcellular localization and interacting protein scaffolds (2). The increasing number of Rab family members during evolution reflects ongoing specialization of membrane trafficking pathways. At least 63 Rabs have been identified in humans. Among these, Rab5 serves as the master regulator of the endocytic trafficking (3). It functions through recruitment of specific effector proteins involved in membrane tethering and docking (4-8). The demonstration that at least 20 cytosolic proteins specifically interact with active Rab5 highlights the complexity of the downstream regulation by this GTPase and raises the possibility that Rab5 might also manage other aspects of the endosome function (4,9). A common feature of the Rab family small GTPases is the existence of subgroups of structurally related isoforms sharing a high sequence identity (10). Rab5 subgroup has three isoforms (A, B, and C) (3). A large-scale mRNA expression profiling study from 79 human and 61 mouse non-redundant tissues shows distinct tissue distributions of the Rab5 isoforms, suggesting that the trafficking properties of the early endosomal network in developmentally distinct cell and tissue types is likely fine-tuned to fulfill sub-specializations of a given pathway (10). In fact, several lines of evidence indicate that Rab5 isoforms can be functionally different. First, Rab5A is found to be the only isoform transcriptionally up-regulated in response to treatment with cytokines like IL-4 or interferon (IFN-γ) in macrophages (11,12). Moreover, Rab5A but not Rab5C was found to be involved in the maturation of phagosomes containing *Listeria monocytogenes* (13). In primary hippocampal

cultures, treatment with DHPG, a group I metabotropic glutamate receptor agonist, leads to up-regulation of Rab5B but not Rab5A, thereby reducing N-methyl-d-aspartate (NMDA)-type glutamate receptor-mediated membrane current and cell death (14,15). Also, Rab5 isoforms can be differentially phosphorylated at a consensus motif in position 123 by distinct proline-directed Ser/Thr kinases *in vitro* (16).

Epidermal growth factor receptor (EGFR) is the prototype of Class I transmembrane receptor tyrosine kinase operating through activation of its intrinsic tyrosine kinase upon ligand binding. Activated EGFR stimulates numerous signal transduction pathways that mediate a wide spectrum of cell responses, including cell proliferation, differentiation and apoptosis (17,18). In order to regulate the strength and duration of the signaling, activated EGFR also initiates a negative-feedback mechanism that eventually leads to the removal of EGF-EGFR complexes from the plasma membrane by endocytosis (19). Previous studies have shown that over-expression of Rab5A enhances EGF-stimulated fluid-phase endocytosis and EGF-EGFR internalization; whereas dominant negative Rab5 represses these processes (20,21). In addition, dominant negative Rab5 substantially inhibits the degradation of EGFR (21). On the other hand, continual expression of constitutively active Rab5 causes a ligand-independent redistribution of EGFR into the intracellular vesicles (22). Interestingly, although expression of a constitutively active Rab5 shows no significant effect on EGFR levels (22), it enhances cell growth and receptor signaling (23).

In this study, we took advantage of the RNAi-silencing technique to individually knock down endogenous Rab5 isoforms in HeLa cells, so that we could examine the loss-of-function phenotypes more likely to capture their physiological activities. Our results indicate that suppression of Rab5A significantly impairs the degradation of EGFR; whereas Rab5C suppression has little effect. The delay of EGFR degradation elicited by the absence of Rab5A occurs after EGFR is internalized, since the rate of EGFR internalization is unaffected by suppression of a single isoform. In addition, we identified Rin1, a Vps9 domain-containing Rab5 exchange factor, to preferentially associate Rab5A, and selectively potentiate its activity in response to EGF stimulation.

EXPERIMENTAL PROCEDURE

Antibodies and Reagents- A mouse monoclonal anti-Rab5A antibody was received as a gift from Dr. A. Wandinger-Ness (University of New Mexico. Albuquerque, NM). Anti-Rab5B antibody was kindly provided by Dr. David B. Wilson (Washington University, St. Louis, MO). Anti-Rab5C antibody is obtained from Sigma Aldrich (Prestige Antibodies®, HPA003426). Human epidermal growth factor and monoclonal anti-EGFR (Ab5) antibody were obtained from CALBIOCHEM. Rabbit polyclonal anti-EGFR (1005) and monoclonal GFP antibody (sc-9996) was from Santa Cruz Biotechnology, Inc. Polyclonal GFP antibody was kindly provided by Dr. Phyllis Hanson (Washington University, St. Louis, MO). Monoclonal anti-V5 antibody was from Invitrogen. Rabbit polyclonal Hrs antibody was a gift of Dr. Tim McGraw (Cornell University). Monoclonal anti-EEA1 and anti-Rin1 antibodies were both from BD Transduction Laboratory. Anti-Cbl antibody (7G10) was from Upstate. Anti-Grb2 antibody was from Cell signaling. Anti-Ubiquitin antibody was obtained from Zymed Laboratories (Invitrogen).

Plasmid constructs- cDNA of Rab5A, B and C were subcloned into SalI/ BamHI sites of pEGFP-C1 (Clontech) and SacI/ BamHI sites of RFP-C3 expression vector (kindly provided by Arnold Hayer et. al., Swiss Federal Institute of Technology (ETH) Zürich, Switzerland). A Rin1 expression construct was prepared by subcloning full-length human Rin1 PCR product into the mammalian expression vector pcDNA3.1/V5-His TOPO TA (Invitrogen).

*siRNA construction and transfection-*The siRNAs against Rab5 isoforms were constructed and purified using the Silencer™ siRNA construction kit (Ambion, Austin, TX) as described. The sequences specific for human Rab5A are 5'-GAGTCCGCTGTTGGCAAATCA-3' (#84) and 5'-AACCAGGAATCAGTGTTGTAG-3' (#623), for human Rab5B are 5'-ACCCAGTCCGTTTGTCTAGAT-3'(#174) and 5'-AAGACAGCTATGAACGTGAAT-3' (#492),

for human Rab5C are 5'-ACCA ACACAGATACATTTGCA-3' (#309) and 5'-AATGAACGTGAACGAAATCTT-3' (#503), and for human Rin1 are 5'-AACAGTCTGAGACAACTGCTG-3' and 5'-AACATGTCCTGGAGAAGTCAT-3'. A scrambled siRNA (Ambion, Austin, TX) or siRNA designed against GFP was used as negative controls. The transfection of siRNA (20 nM final concentration) was performed using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instruction.

Cell culture and transfection- HeLa, HEK293T, and DU145 cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% bovine growth serum (Hyclone Laboratories) containing penicillin and streptomycin. The transfection was performed using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instruction.

Immunoblotting and Immuno-precipitation- Immunoblotting analysis was conducted as described before (1). Briefly, cell lysates were prepared with the lysis buffer containing protease inhibitor cocktail (Sigma). The cell lysates were clarified by centrifugation prior to separation by SDS-PAGE. The resolved proteins were transferred to nitrocellulose membranes (Whatman Schleicher & Schuell, Florham Park, NJ) and then blocked in TBST containing 5% nonfat milk. The membranes were probed with primary antibodies and then HRP-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) and proteins were visualized by enhanced chemiluminescence detection reagents (Pierce). Immunoblot data were quantified by AlphaEaseFC 4.0 software (Alpha Innotech Corp. San Leandro, CA). For immuno-precipitation, the clarified cell lysates were incubated with primary antibodies and protein G-Sepharose (Sigma) overnight at 4 °C. The beads were washed extensively with lysis buffer and solubilized in SDS sample loading buffer.

EGFR Degradation assay- Control or experimental cells were serum starved and then treated with 100 ng/ml EGF and 25 μg/ml cyclohexamide for different times. At the end of each time point, cells were washed with PBS and then lysed in lysis buffer as indicated above. The lysates were subjected to SDS-PAGE and immunoblotting with appropriate antibodies.

*Biotinylation and Internalization of Cell Surface EGF Receptor-*Confluent HeLa cells pre-treated with siRNA were starved in serum-free medium. One set of experiment was kept un-stimulated, while the other two sets were stimulated with 100 ng/ ml EGF for 2 and 5 minutes. The internalization was stopped by washing with ice-cold PBS-Ca-Mg (PBS, pH 7.4, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$). Chilled cells were then incubated with biotin-labeling solution containing 0.5 mg/ml EZ-Link® Sulfo-NHS-LC-Biotin (Pierce) in PBS-Ca-Mg at 4 °C for 30 min. The reaction was quenched by washing the cells with ice-cold PBS-Ca-Mg containing 15 mM glycine. The cells were then washed again with PBS and lysed as described above. The biotinylated-EGFRs were purified using MagnaBind™ streptavidin beads (Pierce) according to the product instructions. Precipitated biotin-EGFR then were subjected to SDS-PAGE and immunoblot analysis. The rate of internalization is calculated by densitometry of EGFR bands.

Quantification of endogenous Rab5 isoforms- His-Rab5A, 5B and 5C recombinant proteins were purified from bacterial lysates. HeLa and DU145 cell lysates were prepared as described above. 5ug or 10 ug of total proteins from DU145 or HeLa cells respectively were subjected to SDS-PAGE alongside purified recombinant Rab5 isoform standards. Intensity of the protein standard bands was quantified with AlphaEaseFc 4.0 software and plotted against their concentration pre-determined with BCA assay in order to define the linear range of the signal. Band signals from cell lysates were then calculated to determine the ratio of different isoforms.

Immuno-fluorescent Microscopy- Cells grown on cover slips were fixed with 3% paraformaldhyde (Electron Microscope Sciences), permeabilized, and then blocked with goat serum. Following incubation with primary antibodies and then Alexa-Fluor594 or 488-conjugated secondary antibodies, the cover slips were mounted with Fluorescent Mounting Medium (DakoCytomation, Carpinteria, CA). Confocal microscopy was performed using a 63X objective and fluorescein

sets on a MRC1024 (Bio-Rad Laboratories). Uptake of Alexa-488-labeled EGF or Transferrin was performed by first starving cells in serum-free medium for 3 hours or 1 hour at 37°C. Cells were then incubated with cold serum-free medium containing 400 ng/ml fluorescein EGF or 40 μg/ml Transferrin for 1 hour at 4 °C. Endocytosis were initiated by replacing the lignad binding medium with pre-warmed medium. At the end of each time point, cells were rapidly chilled, washed, fixed with PFA and then subjected to immunostaining as described above.

Rab5 activation assay- cDNA of the Rab5-binding domain (R5BD, residues 739-862) of Rabaptin5-pGEX was a kind gift from Dr. Guangpu Li (University of Oklahoma Health Sciences Center). The resulting construct termed pGEX/Rabaptin-5(R5BD) was expressed in DH5α. GFP-Rab5 isoforms were expressed in HeLa cells for 24 hours. Cells were first starved and then treated with 100 ng/ml EGF for 5 minutes. Cells were subjected to lysis for 5 min in the lysis buffer (25 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 0.1% NP-40, 10% glycerol, and protease inhibitor). Aliquots of clarified lysates were incubated with GST-R5BD pre-bound to the glutathione-Sepharose-4B resin for 30 min at 4°C on a rotating mixer. The resin was subsequently rinsed with the lysis buffer, resuspended in SDS sample buffer and then subjected to SDS-PAGE and immunoblot analysis.

Statistical analysis- All experiments presented were repeated a minimum of three times. The data represents the mean ± SE. Student's t test was used to calculate statistical significance.

RESULTS

Depletion of Rab5A in HeLa and DU145 cells leads to delayed EGFR degradation

To study the functional differences of endogenous Rab5 isoforms, we designed siRNAs to specifically silence individual isoforms. At least two independent siRNAs were used to suppress each isoform. Immunoblotting with isoform-specific antibodies confirmed that the suppression was more than 90% from each siRNA, and the isoform-specific siRNAs did not cross-react with other isoforms, as the levels of the other two isoforms were unaffected when one isoform was silenced (Fig.1A). Following siRNA treatment, HeLa cells were assayed for EGFR levels at steady state. We found that cells depleted of Rab5A or 5B consistently exhibited increased level of total EGFR; whereas, loss of Rab5C showed very little effects (Fig.1A). The accumulation of EGFR in cells depleted of Rab5A or 5B suggests that the sorting of EGFR into degradation pathway is likely interrupted. Therefore, we examined the rate of EGFR degradation by stimulating HeLa cells with EGF for different times. The levels of EGFR at early time points (3 and 8 minutes) were not used for quantitative analysis, because we found that the immunoblot signals of EGFR were highly variable among experiments at these time points. The results (collected from 15, 30 and 60 minutes) showed that in HeLa cells, the initial rate of degradation was substantially reduced in Rab5A or 5B-depleted cells; however, between 45-60 minutes, the receptors eventually underwent degradation to an extent similar to the control cells (Fig.1B, C and S1). The degree of delay is more profound with Rab5A depletion than with Rab5B knockdowns. Of all three Rab5 isoforms, Rab5C suppression seems to have the least impact. This result suggests that the degradation of EGFR requires both Rab5A and 5B. It is possible that the two isoforms work in concert or sequence along the degradation pathway, so that only one of the two isoforms present is not sufficient to transport EGFR at normal rates. We also tested the rate of EGFR degradation in DU145 cells. DU145 is a prostate cancer cell line expressing EGFR at much higher level than HeLa cells. In agreement with what we found in HeLa cells, the depletion of Rab5A also leads to more delayed EGFR degradation in DU145 cells (Fig.1D). This finding not only verified that the function of endogenous Rab5 is critical for sorting EGFR into degradation pathway, but also provided new evidence indicating a preferential connection between endogenous Rab5A and the trafficking of EGFR.

Quantification of Rab5 isoforms in HeLa and DU 145 cells

Previously, Bucci *et al.* reported that HeLa cells express all three Rab5 isoforms with Rab5B to be more enriched than the other two (16). To test the possibility that the differential delay of EGFR trafficking caused by individual Rab5

isoform silencing corresponds to the relative isoform abundance, we examined the concentration of Rab5 isoforms in HeLa and DU145 cells using purified recombinant Rab5 isoform proteins as standards. Whole cell lysates from HeLa or DU145 cells were analyzed alongside Rab5 protein standards during SDS-PAGE. Following protein gel transfer, membranes were probed with isoform-specific antibodies and the intensity of the bands from protein standards was plotted against its concentration to demonstrate the linearity of the band signals (Fig.S2A and S2B) and the avidity of the isoform-specific antibodies. Using this method, we were able to estimate the ratio of endogenous Rab5A: 5B: 5C was approximately 1: 1: 2 in HeLa cells and 2: 3: 1 in DU145 cells (Fig.2 A and B). These data show that Rab5A is not the most abundant isoform in either cell line, yet it dominates the endocytic trafficking of EGFR.

Over-expression of Rab5A shows more potency in down-regulating EGFR

We next tested if over-expression of individual Rab5 isoforms has opposite effects on EGFR degradation. HeLa cells were transiently transfected with individual CFP-Rab5 isoforms overnight and EGFR degradation assays were carried out as described above. Our findings indicate that exogenously expressed Rab5A, but neither 5B nor 5C accelerated the degradation of EGFR in HeLa cells (Fig. 3A, B). In accordance with the biochemical analysis, immunostaining of total EGFR in cells transfected with RFP-Rab5 isoforms revealed that Rab5A-expressing cells have weaker EGFR signals, suggesting enhanced down-regulation (Fig. 3C, S3). We did, however, notice the degree of acceleration was relatively mild (Fig. 3A, B). Since the transfection efficiency of Rab5 isoform constructs generally does not exceed 80% in HeLa cells, it is possible that the acceleration of EGFR degradation is masked by untransfected cells. Alternatively, we reasoned that under our experimental conditions, endogenous Rab5 isoforms may have already maximized the rate of trafficking and sequestered the limiting factors participating in this process; therefore, excess Rab5 could not improve the transport much further. On the other hand, the finding with Rab5A expression did coincide with the outcome of its depletion, implicating Rab5A as the primary Rab5 isoform in the EGFR degradation pathway.

Depletion of individual Rab5 isoforms shows no significant effect on EGFR internalization

Next, to identify the sites where Rab5A and/or 5B are most active, we examined if loss of individual isoforms could interfere the internalization of EGFR. Control or Rab5 isoform siRNAs-treated HeLa cells were starved and then stimulated with EGF for the indicated times (Fig. 4A, B). EGFR remaining on the cell surface was biotin-labeled, precipitated by streptavidin beads and analyzed with immunoblotting. The data show that depletion of individual Rab5 isoforms did not inhibit the internalization of EGFR, whereas simultaneous depletion of all three isoforms delayed the internalization by ~50% (Fig. 4A, B). Similar results have been reported previously with ^{125}I-EGF based assay by Sorkin *et al.* (24). Similar to EGFR, the endocytosis of Transferrin (Tfn) receptors was not altered by silencing of individual Rab5 isoforms, while silencing all three isoforms together showed marked reduction of Tfn uptake (Fig. 4C, S4).These data suggest that Rab5 isoforms, though actively participating in the internalization step of receptor trafficking, are functional redundant at this stage.

Rab5A or 5B delayed the exit of EGFR from early endosomal compartments

After internalization, EGFR sorting into the late endosome and degradation in the lysosome are necessary to terminate receptor signaling. c-Cbl-mediated ubiquitylation has been shown to be essential for regulating these events and ensuring proper degradation of EGFR (25,26). Upon EGF stimulation, c-Cbl binds directly to the EGFR via Tyr-1045 and indirectly through the SH3 domain of Grb2, whose recruitment to the EGFR is mediated by phosphorylation at Tyr1068 and Tyr1086 of EGFR (27,28). We therefore asked whether the delayed degradaton of EGFR as a result of Rab5A or 5B depletion correlates with altered EGFR phosphorylation and ubiquitylation. By immunoprecipitating EGFR after stimulation for different periods of time and probing ubiquitylated EGFR with anti-ubiquitin antibody, we found that the relative levels of EGFR ubiquitylation over time is not altered by depletion of Rab5A or 5B (Fig. 5A). Consistently, depletion

of Rab5A or 5B did not suppress the phosphorylation of EGFR at Tyr-1045 or Tyr1068 (Fig. 5B, S5), nor did the silencing of Rab5 isoforms interfere the recruitment of c-Cbl or Grb2. However, it is unclear whether depletion of Rab5A or 5B alters the phosphoryaltion of other tyrosine or serine sites of the EGFR and/or obstructs its access to other key adaptors. Further studies will be needed to reveal a more detailed phosphorylation map of EGFR in response to Rab5 isoform silencing.

To further investigate how the loss of individual isoforms changes endosomal dynamics, we examined the subcellular distribution of EEA1, a FYVE-domain containing protein known to associate with the early endosomal compartment (4,6,29) and Hrs, that also localizes on early endosomal membranes and transfer cargos with ubiquitin moieties to downstream multivesicular body sorting machineries (30). In the mean time, the transport of EGFR was visualized by either indirect EGFR immunostaining or by using Alexa488-labeled EGF. Consistent with the immunoblotting data, we observed stronger EGF (or EGFR) signals in Rab5A or 5B-depleted cells (Fig.6). The association of EEA1 with membrane structures was not impaired by loss of individual Rab5 isoforms, nor was the distribution of Hrs and CD63, a late endosomal marker (Fig.6 and S6). Similar to control cells, the EGF-EGFR complex in isoform-silenced cells entered EEA1 positive compartments within 10 minutes (Fig.6A). At later time points, most EGFR containing vesicles was still EEA1 positive in Rab5A- or 5B- silenced cells, while a substantial subset of EGFR in control and Rab5C-depleted cells had exited from early endosomal compartments (Fig.6B and C). Similar results were obtained with Hrs co-immunostaining (Fig.S6A). 45 minutes after endocytosis, EGFR signals had almost completely disappeared from Hrs-positive compartments in control or Rab5C knock-down cells, but still partially co-localized with Hrs in Rab5A, or 5B knock-down cells (Fig.S6A). These results suggest that depletion of Rab5A or 5B slows down the progression of EGFR from early to late endosomal compartments, thereby causing the delay of EGFR degradation. Even so, Rab5A or 5B-silenced cells did eventually complete the degradation of EGFR, suggesting that they can still overcome this blockage presumably via residual activity from incompletely silenced Rab5 isoform, compensatory activities from Rab5 isoforms not targeted by siRNA or alternative mechanisms independent of Rab5.

Rin1 preferentially associates with Rab5A, which facilitates the activation of Rab5A in response to EGF stimulation

Previous studies have shown that Rab5 can be activated by HGF stimulation (31) and that active Ras, a consequential effector of EGFR signaling, potentiates the activity of Rab5A *in vitro* (32). Both studies suggest that the function of Rab5 can be regulated by receptor signaling. In an effort to better understand why Rab5A displays more potency in the EGFR degradation pathway, we tested if Rab5 isoforms are differentially activated in response to EGF stimulation. We took advantage of a Rabaptin-5 Rab5 binding domain (R5BD)-based GST pull down assay (33) to study the activation Rab5 isoforms *in vivo*. We found that after 5 minutes of EGF treatment, more Rab5A was bound to GST-R5BD, indicating more activation of Rab5A; whereas, Rab5B and Rab5C activation did not increase in response to EGF at this time point (Fig. 7). This result further supports the hypothesis that Rab5A is more potent in regulating the trafficking of EGFR as its activation is more responsive to the EGF stimulation. Our previous work shows that Rin1, a Vps9 domain-containing Rab5 exchange factor, relays EGFR signals to Rab5 activation (32). Also, Rin1 has an SH2 domain that couples Rab5 activation to the proximity of EGFR (34). Recently, we found that Rin1 works in concert with ESCRT-0 complex (STAM1/Hrs) in down-regulation of EGFR (1). These data make Rin1 a prime target in Rab5-mediated EGFR trafficking. Therefore, we tested if Rin1 shows any specificity towards Rab5 isoforms. Dominant negative (S34N), and GTP hydrolysis-deficient constitutively active (Q79L) forms of Rab5 isoforms were used to evaluate Rin1 specificity. We carried out a co-immunoprecipitation study with cells transiently co-expressing one of the Rab5 mutant isoforms along with Rin1. Characteristic of exchange factors, Rin1 binds preferentially to dominant negative Rab5, which is primarily GDP-bound. Interestingly, we found that Rin1 bound to Rab5A with higher affinity than to Rab5B or Rab5C (Fig. 8A). Another Rab5 exchange factor, GAPex-5

(human RME-6) (35), was also tested for Rab5 isoform specificities, and no apparent selectivity was found (data not shown). To determine if the activation of Rab5A by EGF stimulation is Rin1-dependent, HeLa cells transfected with Rab5A and scramble or Rin1-specific siRNA were subjected to R5BD-pull down assay as described above. We found that in the absence of Rin1, the activation of Rab5A in response to EGF stimulation is inhibited (Fig. 8B). These results suggest that Rab5 isoforms may be differentially regulated by various exchange factors, whose abundance, subcellular localization and responses to extracellular stimulation can potentially direct their favored isoforms to regulate certain pathways, but not others.

DISCUSSION

The focus of this study is to investigate the functional significance of each Rab5 isoform. Work from our lab and others has demonstrated the many facets of Rab5 in regulating endocytosis, signal transduction and cytoskeletal dynamics (36-39). Here, we provide new evidence suggesting that Rab5 isoforms have distinct capacity to coordinate the trafficking of EGFR, rather than acting only as redundant mechanisms

Rab5A is the predominant isoform in EGFR degradation pathway

After effective down-regulation of Rab5 isoforms with siRNAs, we observed that the trafficking of EGFR was delayed by Rab5A and to a lesser extent by 5B suppression, but barely affected by Rab5C silencing in both HeLa and DU145 cells. This result suggests that both endogenous Rab5A and 5B take part in EGFR trafficking, though their efficacy in mediating this process and sites of action may vary. We then looked into the expression level of endogenous Rab5 isoforms in HeLa and DU145 cells and found that Rab5A is no more enriched than Rab5B or Rab5C, indicating that the delayed degradation could not simply be attributed to overall reduction of Rab5 levels in cells. The retardation of EGFR trafficking was further analyzed by light microscopy to clarify the subcellular compartments retaining EGFR in the absence of each Rab5 isoform. We showed that EGFR entered early endosomal compartments positive for EEA1 within 10 minutes of internalization regardless of Rab5 isoform depletion. At later time points, most of the receptors had progressed out of EEA1- or Hrs-positive vesicles in control and Rab5C-depleted cells, yet remained partially localized to early endosomal compartments in Rab5A- and Rab5B-depleted cells. Though the loss of Rab5A or 5B impaired the trafficking of EGFR, it did not appear to alter the normal covalent modifications of EGFR essential for its proper sorting. Taken together, following the down-regulation of the endogenous Rab5 isoforms, we observed a differential contribution of Rab5 isoforms in EGFR trafficking. In agreement with silencing results, over-expression of Rab5A appeared to accelerate EGFR trafficking more effectively than other isoforms. In fact, previous work from our lab has also demonstrated a close connection between Rab5A and EGFR-related pathways. Barbieri *et al.* have shown that ^{125}I-EGF internalization and EGF-stimulated HRP uptake in fibroblasts are more up-regulated by over-expression of Rab5A compared with Rab5B or 5C (20). It is to be noted that the phenotypes of Rab5B suppression and overexpression did not coincide. In the absence of Rab5B, EGFR degradation is moderately impaired, but overexpression of Rab5B hardly strikes any difference. One possible explanation is that the type of cells we used for overexpression does not have sufficient and appropriate combinations of Rab5B regulators and/or effectors to transduce its activity into phenotypes; whereas, RNAi ablation of Rab5B directly perturbs the homeostasis maintained by endogenous Rab5B, which is more easily detected. We propose that endogenous Rab5B may work in conjunction or in sequence with Rab5A to facilitate the trafficking of EGFR. Interestingly, when we employed an *in vitro* system to evaluate the strength of Rab5 isoforms in promoting vesicle fusion, our preliminary data indicated that purified recombinant Rab5A was more potent in activating endosome-endosome fusion, and loss of endogenous Rab5A in cell membrane fractions lead to decreased fusion activities (data not shown). This result not only further supports the concept of differential potency from Rab5 isoforms, but also suggests that Rab5B participates in EGFR trafficking in a different manner. Recent studies point to a role of Rab5-GTP in coordinating membrane tethering and

fusion with cytoskeletal-based organelle motility (9,40) . Spatial trafficking of the early endosomes from the cell periphery to the cell center is critical for their ability to generate endosomal carrier vesicles that bud from early and fuse with late endosomes or mature into late endosomes (40,41). It is possible that Rab5B, instead of promoting endosome-endosome fusion, governs this aspect of the EGFR trafficking. In fact, we did, on multiple occasions, notice internalized EGF-EGFR vesicles to be scattered to sites more adjacent to the plasma membrane in Rab5B knockdown cells than in control or Rab5A knockdown cells. These data suggests that after EGFR is internalized, the inward movement of vesicles is likely impeded when Rab5B is repressed. More studies will be needed to clarify the involvement and activity of different Rab5 isoforms in modulating the motility of endosomes.

Rab5A preferentially interacts with Rin1, a multi-domain guanine nucleotide exchange factor that binds EGFR and regulates the EGFR degradation

The question remains unclear as to what causes the difference among Rab5 isoforms in the EGFR degradation pathway. Overexpressed Rab5 isoforms in cells have similar subcellular localization, suggesting that all three isoforms are properly targeted to membrane structures and Rab5A is more active in EGFR trafficking pathway not because of better membrane association. However, it is yet to be determined whether endogenous Rab5 isoforms are localized differently. Another possibility would be differential regulation of the isoform activities by GEFs and GAPs. At least nine different Rab5 GEFs have been identified and seven of them are proven to have GEF activity *in vitro* (7,8,32,35,42-45). In addition to vesicle fusion, Rab5 can also control the motility of organelles along microtubules (9,40), actin remodeling (37-39), and participation in proliferative cell signaling pathways (23,36). It is reasonable to speculate that the wide range of Rab5 exchange factors contribute to the complexity of these proposed functions. Indeed, we tested the binding specificity of some of these GEFs towards Rab5 isoforms, and found that Rin1 preferentially binds to Rab5A, whereas GAPex-5 showed no significant specificities towards isoforms. As we tested the activation of Rab5 isoforms in response to EGFR stimulation, Rab5A displayed strong and acute activation, which is in good correlation with its potency in EGFR trafficking. More importantly, the activation of Rab5A upon EGF stimulation is inhibited by silencing of Rin1. That is, the preferential interaction between Rab5A and Rin1 appears to control the ability and sensitivity of Rab5A in regulating EGFR degradation pathway in HeLa cells. However, it remains unclear whether the preferential interaction between Rin1 and Rab5A selectively regulates the trafficking of only a certain receptors. Barbieri *et al.* showed that Rin1 associates with several signaling receptors, but not cargo receptors, such as Tfn receptor and Mannose receptor (34). Moreover, overexpression of Rin1 does not alter the uptake of Tfn, nor was the recycling of Tfn in HeLa cells affected by Rin1 depletion (Fig.S7). These results suggest that Rin1 may provide selectivity for Rab5A in regulation of the trafficking of specific receptors.

As more functions have been shown to involve Rab5, it is possible that different Rab5 isoforms play more significant roles in distinct functions. In fact, preliminary studies examining Rab5-mediated cell migration implicates Rab5C to be more engaged to cell migratory activities than other Rab5 isoforms (Chen and Stahl, unpublished data). In agreement with this finding, a recent report indicated that Rab5C is the only isoform expressed during gastrulation of zebrafish and mediates Wnt11-controlled mesendodermal cell cohesion and migration (46). Ulrich *et al.* proposed that Wnt11 promotes Rab5C-mediated endocytosis and recycling of E-cadherin to regulate the dynamics of E-cadherin turnover at the plasma membrane. Alternatively, Wnt11 might regulate Rab5-dependent actin remodeling, which in turn could affect the adhesive and cohesive properties of mesendodermal cells.

It is important to point out that lower eukaryotic model organisms such as *Drosophila* and *C. elegans* have only one Rab5 paralogue, and loss of its function has been shown to result in embryonic lethality (47,48); whereas, three isoforms are identified for higher organisms (i.e. human and zebrafish). The multiplication of Rab5 paralogues from its ancestral origin implicates continued evolution and specialization of the early endosomal systems. The most defining biological attributes of higher organisms are the development

of nervous and immune systems, both of which require highly organized endocytic networks in order to manage the intricacy of their respective functions. In the nervous system, not only Rab5 is shown to associate with vesicles involved in cycling of neurotransmitters during synaptic transmission (47), but its activity appears to initiate neuronal cell differentiation and dendrite branching (33,49,50), and participates in the delivering of neurotrophic "signaling endosomes" via dynein and kinesin (50,51). Several neurodegenerative diseases are linked to faulty axonal transport (52). The juvenile amyotrophic lateral sclerosis-associated gene ALS2, encoding the protein Alsin, whose Vps9 domain regulates the activation of Rab5 (43). Depletion mutation of ALS2 within its Vps9 domain has been identified to cause hereditary spastic paraplegia (53), further stressing the role of Rab5 in neuronal pathogenesis. Recent evidence also revealed multiple endocytic mechanisms governing antigen presentation (54). Depending upon the nature of the antigens as well as the endocytosis receptors, antigens can be routed to phagosomes or endosomes for peptide processing and loading. The molecular basis that assigns each Rab5 isoform to a specific function is still unclear. In this study, we provided new evidence suggesting that Rab5 isoforms have preferences towards their exchange factors and certain pathways. Given the need for flexible yet precise regulation of these neuronal and immune responses, it is reasonable to anticipate that Rab5 isoforms differentially integrate their binding specificities and/or tissue distribution into the regulation of multiple pathways. It will be of great importance to dissect the roles of Rab5 isoforms in these pathways by means of individual Rab5 isoform ablation in the future.

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FOOT NOTES

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The abbreviations used are: Rin1, Ras interaction/interference1; EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; STAM, signal transducing adapter molecule; EEA1 early endosomal antigen 1; Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate; ESCRT, endosomal sorting complex required for transport; siRNA, short interfering RNA; GFP, green fluorescent protein; SH, Src homology; GEF, Guanine nucleotide exchange factor; GAP, GTPase activating protein.

FIGURE LEGENDS

Figure 1. Depletion of individual Rab5 isoforms differentially delayed the degradation of EGFR in HeLa and DU145 cells

A) HeLa cells were transfected with 20 nM siRNA targeting GFP (as negative control) or specific sequences of the Rab5 isoforms. 48 hours post-transfection, cells were harvested and then subjected to SDS-PAGE and immunoblot analysis. Proteins were probed with specific antibodies as indicated. B) HeLa cells transfected with siRNA targeting GFP (as negative control), Rab5A, Rab5B or Rab5C were

starved for 2-3 hours and degradation of EGFR was stimulated with 100 ng/ml EGF for the indicated times. Cell lysates were subjected to SDS-PAGE analysis. Proteins were probed with specific antibodies as indicated. C) Band intensity from 15, 30 and 60 minutes was quantified with AlphaEaseFc 4.0 software. The graph was acquired from three independent experiments. The data represent the mean value ± S.E. * $P<0.007$ compared to control. D) DU145 cells were transfected with siRNA targeting GFP (as negative control), Rab5A, Rab5B or Rab5C. 24 hours post-transfection, cells were starved overnight and degradation of EGFR was stimulated with 100 ng/ml EGF in the presence of cycloheximide for 5 hours. Cell lysates were processed as described above. Proteins were probed with specific antibodies as indicated. Band intensity was quantified with AlphaEaseFc 4.0 software. The graph was acquired from three independent experiments. The data represent the mean value ± S.E. * $P<0.006$ compared to control

Figure 2. Determination of the abundance of endogenous Rab5 isoforms in HeLa and DU145 cells

5 or10 ug of total cell lysates from siRNA-treated DU145 or HeLa cells were subjected to SDS-PAGE analysis alongside purified His-Rab5 isoforms ranging from 1.25 ng to 10 ng as standards. Proteins were probed with isoform specific antibodies and developed with ECL. Band intensity was quantified with AlphaEaseFc 4.0 software (supplementary figure 2). A) The experiment was repeated three times. The graph shows the relative abundance of the three isoforms in HeLa cells. The data represents the mean value of three independent experiments ± SE. B) The abundance of Rab5 isoforms in DU145 was determined as described above. The graph shows the relative abundance of the three isoforms in DU145 cells. The data represent the mean value of three independent experiments ± SE.

Figure 3. Over-expression of Rab5A accelerates the trafficking of EGFR

A) HeLa cells were transfected with CFP, CFP-Rab5A, 5B, or 5C. 24 hours post-transfection, cells were starved and then subjected to EGFR degradation assay. Cell lysates were analyzed by SDS-PAGE. Proteins were probed with specific antibodies as indicated. B) Band intensity was quantified with AlphaEaseFc 4.0 software. The graph was acquired from three independent experiments. The data represent the mean value ± SE. C) HeLa cells were transfected with RFP-Rab5A, Rab5B, or Rab5C. 24 hours post-transfection, cells were fixed and immunostained with EGFR antibody to determine the level of EGFR in cells. Scale bar represents 5 μm

Figure 4. Internalization of EGFR and Transferrin receptors are not inhibited by individual Rab5 isoform depletion

A) The rate of EGFR internalization was examined by labeling cell surface EGFR with biotin after stimulation briefly with EGF. Magnetic streptavdin beads then were used to pull-down biotin labeled surface EGFR. After washes, bound EGFR were released from the beads with SDS sample buffer, and then analyzed with SDS-PAGE. B) The intensity of the bands were quantified with AlphaEaseFc 4.0 software and plotted as percentage of EGFR remaining on cell surface over time. The data represent the mean value of three independent experiments ± SE. C) Control or specific-siRNA transfected cells were starved for one hour to deplete endogenous Tfn before the uptake assay. Next, cells were pre-bound with Alexa-488-Tfn for 1 hour at 4°C. Internalization was then initiated by replacing the binding medium with pre-warmed internalization medium and incubating at 37°C for 5 minutes. At the end of uptake, cells were washed and fixed for confocal microscopy analysis. Scale bar represents 10 μm

Figure 5. Phosphorylation and ubiquitylation of EGFR is not altered by silencing of Rab5 isoforms

A) Control or Rab5 isoform-silenced HeLa cells were starved and stimulated with EGF at 37°C for the indicated times. Cell lysates were then subjected to EGFR immunoprecipitation and immunoblotting with pan-ubiquitin antibody to detect the ubiqitylated EGFR. Blots were re-probed with EGFR antibody to show the total EGFR levels in the immunoprecipitates. B) Control or Rab5 isoform-silenced cells were

starved and then stimulated with EGF at 4 °C for one hour. Cell lysates were then subjected to EGFR immunoprecipitation and immunoblotting with antibodies as indicated. Co-immunoprecipitated adaptor proteins were detected with specific antibodies as indicated.

Figure 6. Trafficking of EGFR into EEA1-positive compartments is not altered by depletion of Rab5 isoforms, but exit from early endosomal compartments is delayed
HeLa cells were transfected with control or Rab5 isoform-specific siRNA. 48 hours post-transfection, cells were starved, incubated with Alexa488-EGF for 1 hour at 4 °C, and then allowed to endocytose for A) 10 minutes, and B) 25 minutes at 37 °C. Cells were then fixed and immunostained for EEA1 (2° Ab: Alexa594-IgG). Scale bar represents 10 μm C) Colocalization between EGF/EGFR and EEA1 was quantified with ImageJ and the mean value of the Pearson's coefficient ± SE from 7 images per condition is illustrated in the graph.

Figure 7. Rab5A is activated in response to EGF stimulation
HeLa cells were transfected with GFP-Rab5A, 5B or 5C. 24 hours post-transfection, cells were starved for 3 hours and then stimulated with EGF for 5 minutes. Cell lysates were collected and subjected to R5BD-GST pull down assay as described in Experimental Procedure. Bound GFP-Rab5 isoforms were then analyzed with SDS-PAGE and immunoblotting. B) Band intensity from blots was quantified, and normalized to total GFP-Rab5 isoform signals from whole cell lysates (WCL). The adjacent graph represents mean ± SE of the relative activation of Rab5 isoforms from three independent experiments.
* $P < 0.04$

Figure 8. Rin1 preferentially binds Rab5A to facilitate the degradation of EGFR
A) HEK293 cells were co-transfected with V5-tagged Rin1 along with constitutively active (Q79L) or negative (S34N) mutants of GFP-Rab5A, Rab5B or Rab5C. Cell lysates were pre-cleared with centrifugation and then immuno-precipitated with anti-GFP antibody. Immunoprecipitates were then separated by SDS-PAGE and blotted with antibodies as indicated above. Total lysates were also analyzed to show the expression level of recombinant proteins in each sample. B) HeLa cells were co-transfected with GFP-Rab5A and scramble- or Rin1-targeting siRNA. 24 hours post-transfection, cells were starved for 3 hours and then stimulated with EGF for 5 minutes. Cell lysates were collected and subjected to R5BD-GST pull down assay as described in Experimental Procedure. Bound GFP-Rab5 isoforms were then analyzed with SDS-PAGE and immunoblotting. C) Band intensity from blots was quantified, and normalized to total GFP-Rab5A signals from WCL. The adjacent graph represents mean ± SE of the relative activation of Rab5A from three independent experiments.

Figure 1

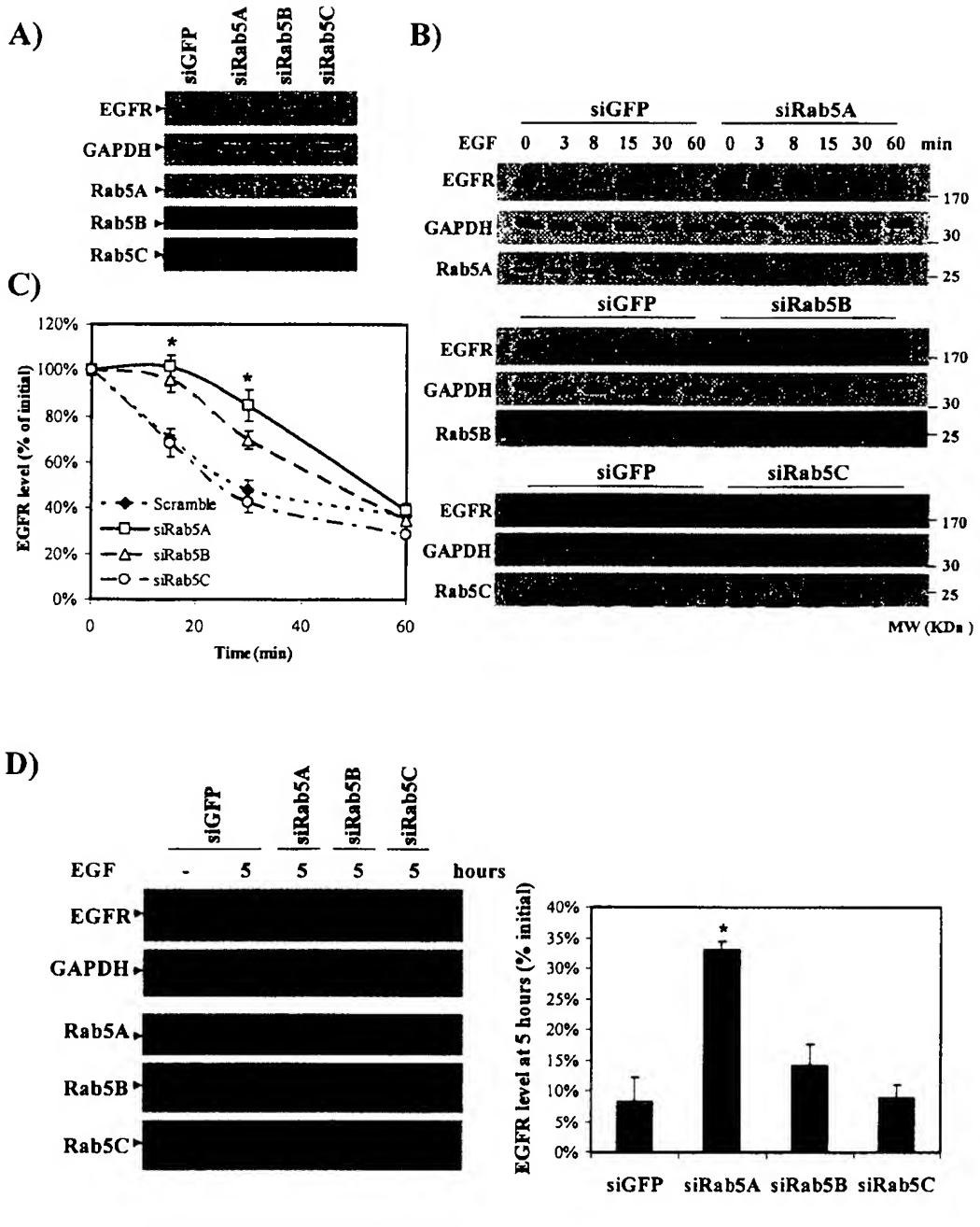

A)
siGFP
siRab5A
siRab5B
siRab5C
EGFR
GAPDH
Rab5A
Rab5B
Rab5C
B)
siGFP
siRab5A
EGF 0 3 8 15 30 60 0 3 8 15 30 60 min
EGFR
GAPDH
Rab5A
170
30
25
siGFP
siRab5B
EGFR
GAPDH
Rab5B
170
30
25
siGFP
siRab5C
EGFR
GAPDH
Rab5C
170
30
25
MW (KDa)
C)
120%
100%
80%
60%
40%
20%
0%
EGFR level (% of initial)
Scramble
siRab5A
siRab5B
siRab5C
0
20
40
60
Time (min)
D)
siGFP
siRab5A
siRab5B
siRab5C
EGF - 5 5 5 5 hours
EGFR
GAPDH
Rab5A
Rab5B
Rab5C
EGFR level at 5 hours (% initial)
40%
35%
30%
25%
20%
15%
10%
5%
0%
siGFP
siRab5A
siRab5B
siRab5C

Figure 2

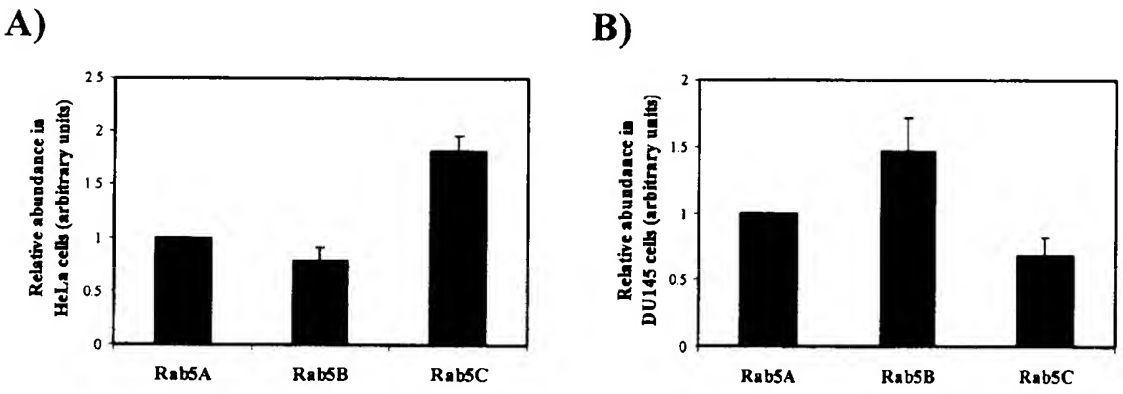

A)
Relative abundance in HeLa cells (arbitrary units)
2.5
2
1.5
1
0.5
0
Rab5A
Rab5B
Rab5C
B)
Relative abundance in DU145 cells (arbitrary units)
2
1.5
1
0.5
0
Rab5A
Rab5B
Rab5C

Figure 3

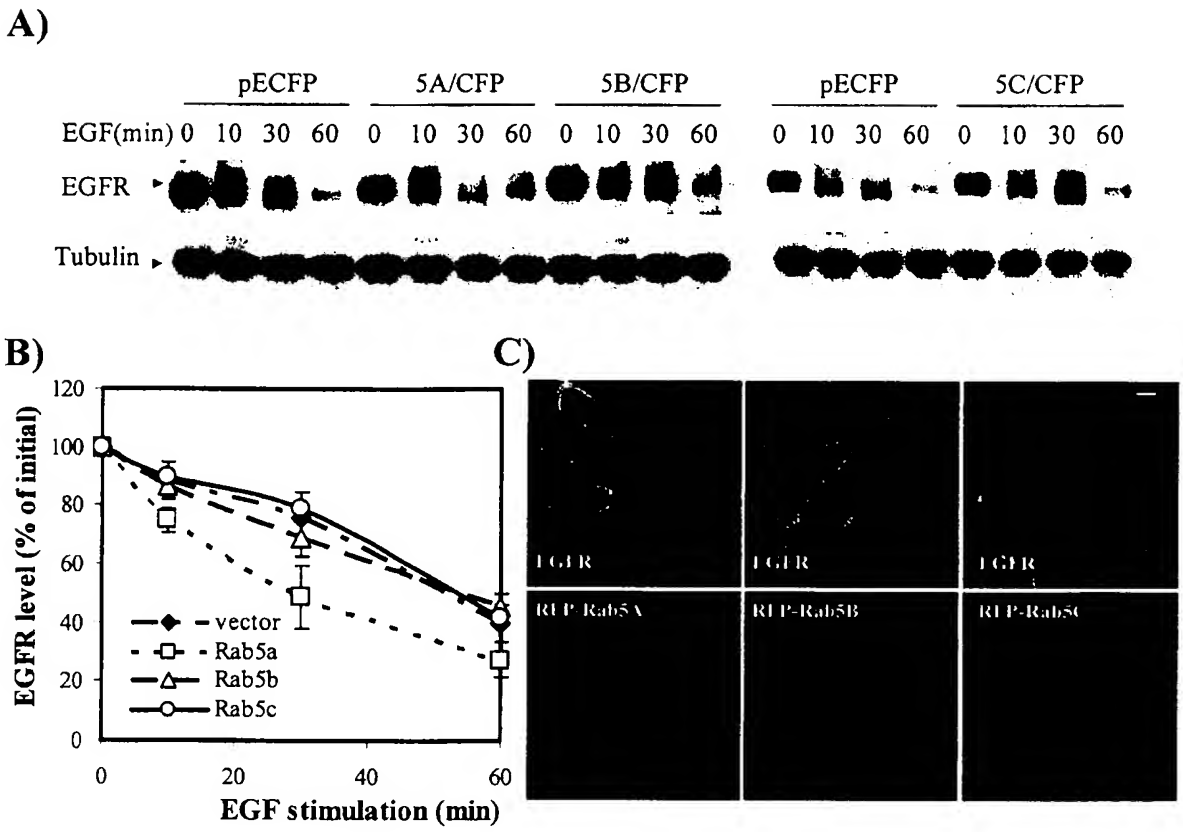

A)
pECFP
5A/CFP
5B/CFP
pECFP
5C/CFP
EGF(min) 0 10 30 60 0 10 30 60 0 10 30 60 0 10 30 60 0 10 30 60
EGFR
Tubulin
B)
EGFR level (% of initial)
120
100
80
60
40
20
0
0
20
40
60
EGF stimulation (min)
vector
Rab5a
Rab5b
Rab5c
C)
EGFR
EGFR
EGFR
RFP-Rab5A
RFP-Rab5B
RFP-Rab5C

Figure 4

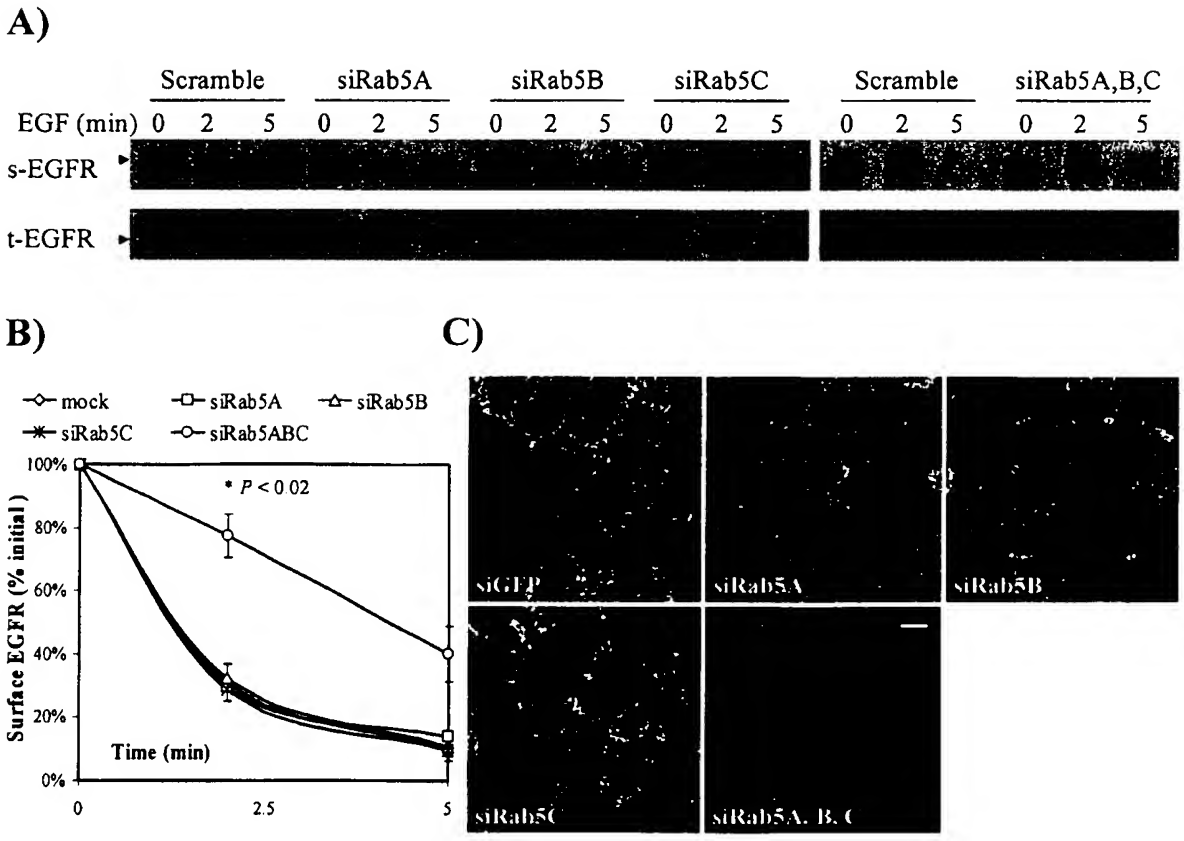

A)
Scramble
siRab5A
siRab5B
siRab5C
Scramble
siRab5A,B,C
EGF (min) 0 2 5 0 2 5 0 2 5 0 2 5 0 2 5 0 2 5
s-EGFR
t-EGFR
B)
mock
siRab5A
siRab5B
siRab5C
siRab5ABC
* P < 0.02
Surface EGFR (% initial)
100%
80%
60%
40%
20%
0%
Time (min)
0
2.5
5
C)
siGFP
siRab5A
siRab5B
siRab5C
siRab5A, B, C

Figure 5

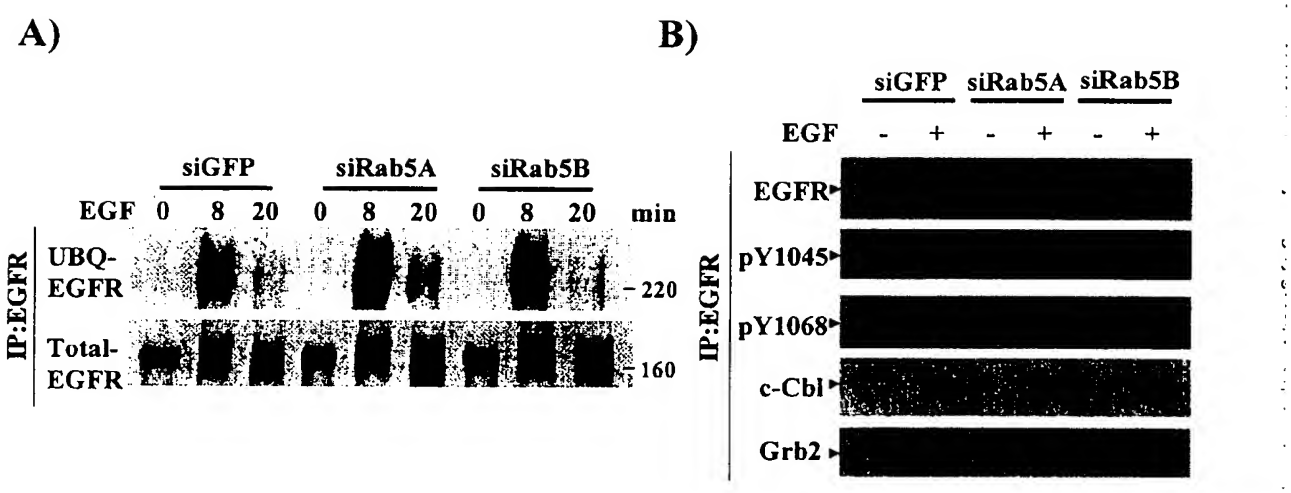

A)
siGFP
siRab5A
siRab5B
EGF 0 8 20 0 8 20 0 8 20 min
IP:EGFR
UBQ-EGFR
Total-EGFR
220
160
B)
siGFP siRab5A siRab5B
EGF - + - + - +
IP:EGFR
EGFR
pY1045
pY1068
c-Cbl
Grb2

Figure 6

A) Alexa488-EGF Uptake: 10 min

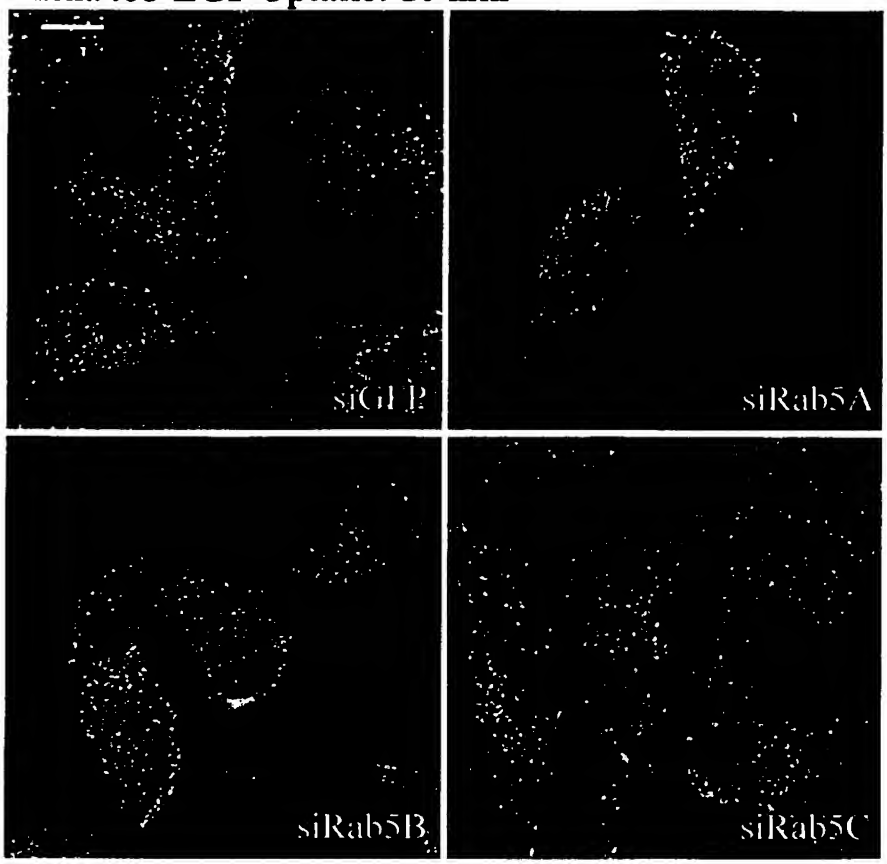


Figure 6

B) Alexa488-EGF Uptake: 25 min

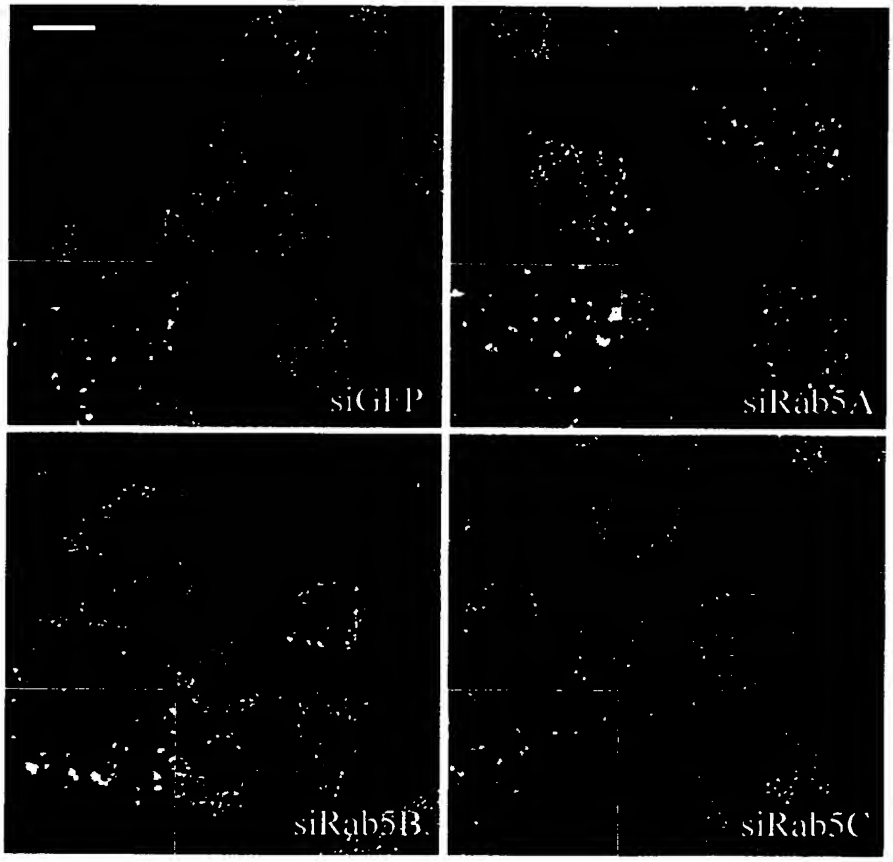


Figure 6

C)

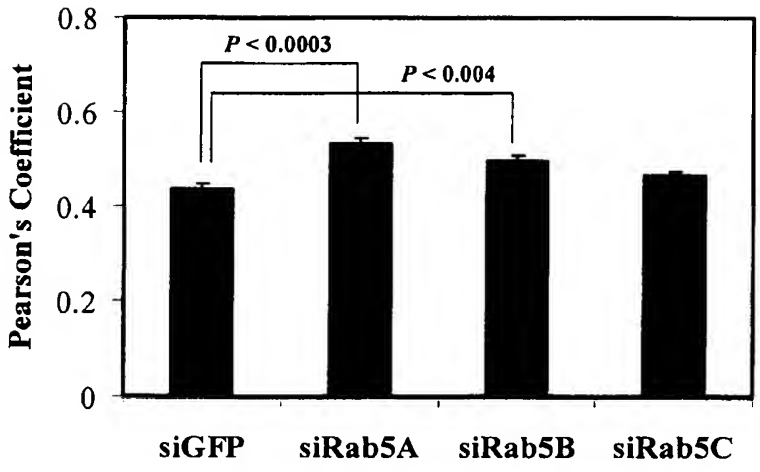

0.8
0.6
0.4
0.2
0
Pearson's Coefficient
P < 0.0003
P < 0.004
siGFP
siRab5A
siRab5B
siRab5C

Figure 7

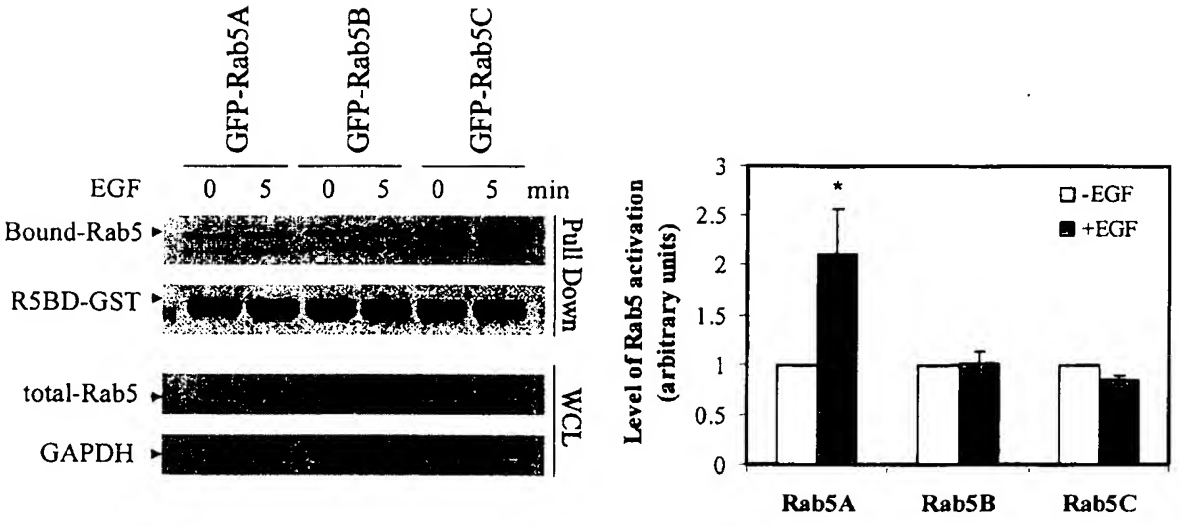
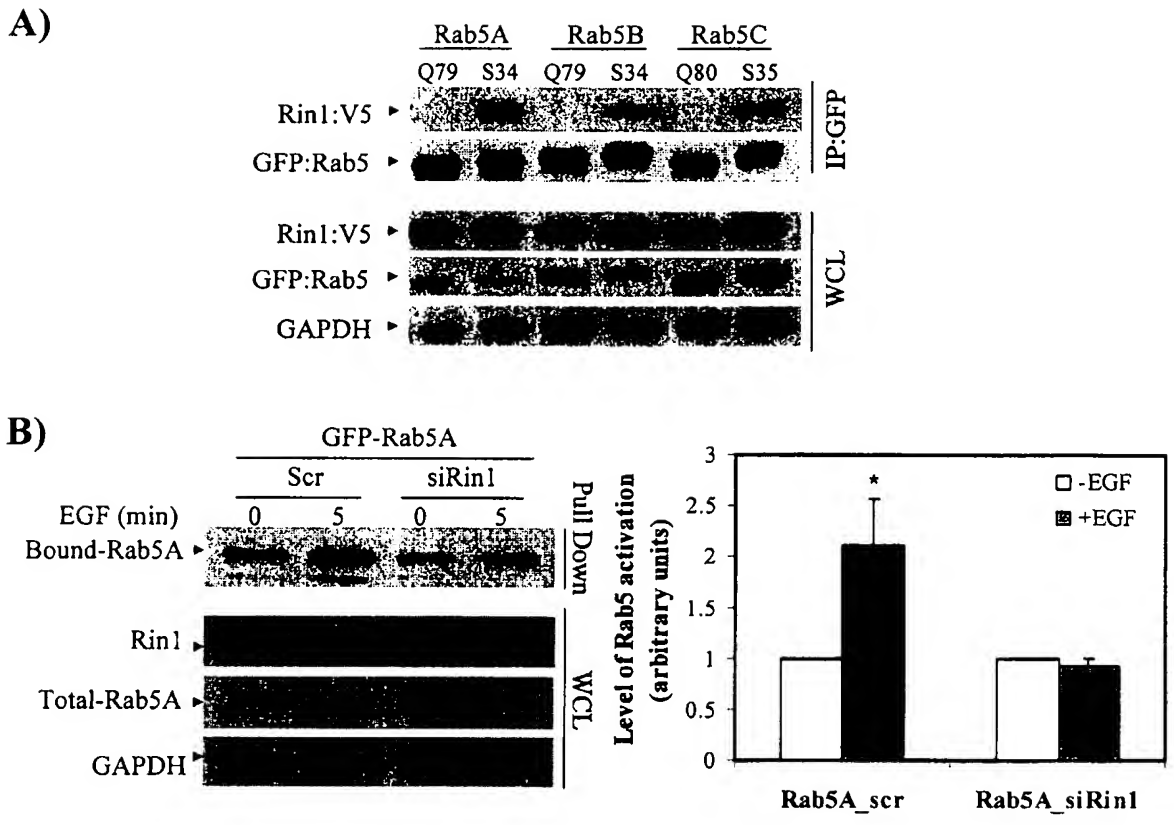

GFP-Rab5A
GFP-Rab5B
GFP-Rab5C
EGF
0 5 0 5 0 5 min
Bound-Rab5
R5BD-GST
Pull Down
total-Rab5
GAPDH
WCL
Level of Rab5 activation (arbitrary units)
3
2.5
2
1.5
1
0.5
0
*
-EGF
+EGF
Rab5A
Rab5B
Rab5C

Figure 8


A)
Rab5A
Rab5B
Rab5C
Q79 S34 Q79 S34 Q80 S35
Rin1:V5
GFP:Rab5
IP:GFP
Rin1:V5
GFP:Rab5
GAPDH
WCL
B)
GFP-Rab5A
Scr
siRin1
EGF (min)
0 5 0 5
Bound-Rab5A
Pull Down
Rin1
Total-Rab5A
GAPDH
WCL
Level of Rab5 activation (arbitrary units)
-EGF
+EGF
Rab5A_scr
Rab5A_siRin1